New Phytologist

Review

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Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes

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Received: *22 March 2006*
Accepted: *23 May 2006*

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Key words: *Blumeria graminis, Colletotrichum, Hyaloperonospora parasitica, Magnaporthe grisea, Phytophthora, Uromyces fabae.*

Summary

'Compatibility' describes the complementary relationship between a plant species and an adapted pathogen species that underlies susceptibility and which ultimately results in disease. Owing to elaborate surveillance systems and defence mechanisms on the plant side and a common lack of adaptation of many microbial pathogens, resistance is the rule and compatibility the exception for most plant–microbe combinations. While there has been major scientific interest in 'resistance' in the past decade, which has revealed many of its underlying molecular components, the analysis of 'compatibility', although intimately intertwined with 'resistance', has not been pursued with a similar intensity. Various recent studies, however, provide a first glimpse of the pivotal players and potential molecular mechanisms essential for compatibility in both the plant and parasite partners. In this review we highlight these findings with a particular emphasis on obligate biotrophic and hemibiotrophic fungal and oomycete pathogens and discuss novel strategies that might help to uncover further the molecular principles underlying compatibility to these highly specialized pathogens.

New Phytologist (2006) **171**: 699–718

doi: 10.1111/j.1469-8137.2006.01829.x

New Phytologist

I. Introduction

Biotrophic fungi are considered to obtain their energy from the living cells of their hosts (Lewis, 1973). Associations between biotrophic fungi and vascular plants range from mutualistic, for example the arbuscular mycorrhiza fungi, through to entirely parasitic, for example the rust and powdery mildew pathogens. Biotrophic plant–fungal associations might have already evolved when the earliest plants colonized the land because the intracellular arbuscules of mycorrhiza fungi have been found in fossil roots dating from the Lower Devonian (400 million years old) (Remy *et al.*, 1994). The capacity to establish biotrophic relationships with host plants has also arisen in highly divergent fungal taxa (Table 1), including the Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, Plasmodiophoromycota and the fungus-like Oomycota (Kingdom Chromista).

Among biotrophic fungi and oomycetes there is considerable variation in the duration of the biotrophic relationship and their capacity for saprotrophic growth *in vitro* or necrotrophic growth on dead plant tissues. For example, hemibiotrophs such as *Magnaporthe grisea*, *Colletotrichum* spp. and *Phytophthora* spp. initially feed biotrophically for varying periods before switching to necrotrophy and can be cultured axenically (Table 1). Facultative biotrophs, for example *Ustilago maydis* and *Claviceps purpurea*, grow entirely biotrophically in nature but are also culturable (Kahmann & Kämper, 2004; Tudzynski & Scheffer, 2004). On the other hand, obligate biotrophs, such as the rusts, powdery mildews and downy mildews, depend on living plant tissue for their growth and reproduction, and are either unculturable or grow only to a limited extent *in vitro* (Fasters *et al.*, 1993, Table 1).

The early steps by which biotrophs establish infection, namely adhesion to the plant surface, germination and the differentiation of penetration structures (appressoria), do not differ greatly from the prepenetration behaviour of necrotrophs and have been reviewed extensively elsewhere (Mendgen *et al.*, 1996; Deising *et al.*, 2000; Tucker & Talbot, 2001). After initial entry, biotrophic pathogens colonize plant tissues by several different routes: their hyphae may spread over the plant cuticle, under the cuticle, between host cells or inside host cells (Table 1). This review is concerned with the most sophisticated biotrophs and hemibiotrophs that establish intimate contact with their hosts by inserting specialized infection structures (intracellular hyphae or haustoria) into living plant cells. We focus particularly on the decisive steps of host cell entry, differentiation of intracellular infection structures and their accommodation by host cells. In addition, we highlight the crucial role that pathogen 'effectors' and plant 'compatibility factors' are likely to play in the establishment of plant–biotroph compatibility. Where appropriate, we refer to bacterial and fungal biotrophic pathogens such as *Pseudomonas syringae*, *Cladosporium fulvum* and *Ustilago maydis* which lack specialized 'intracellular' infection structures, or draw comparisons with intracellular symbionts such as arbuscular mycorrhiza fungi.

II. Plant cell entry control

1. Host cell entry – a milestone towards compatibility

As an integral part of their life cycle, many (hemi-)biotrophic pathogens must enter host cells in order to elaborate haustoria or intracellular hyphae (to be described later). For example, the powdery mildews, anthracnose fungi (*Colletotrichum* spp.) and the rice blast fungus (*M. grisea*) usually initiate their life cycle by direct invasion of leaf epidermal cells, while biotrophic oomycetes and dikaryotic rust fungi typically penetrate leaf mesophyll cells from an intercellular mycelium. In both cases, initial host penetration frequently depends upon the formation of appressoria at appropriate locations on the plant surface, for example in response to host topographical cues such as stomatal pores and anticlinal cell walls (reviewed by Read

Table 1 Biotrophic lifestyles of some fungal and oomycete plant pathogens

Mode of host colonization	Pathogen and type of biotrophy
Subcuticular hyphae	Ascomycota: *Venturia* (H), *Phyllosticta* (H), *Pyrenopeziza* (H), *Diplocarpon* (H)
Epicuticular hyphae with intracellular haustoria	Ascomycota (powdery mildews): *Erysiphe* (O), *Blumeria* (O), *Oidium* (O)
Intercellular hyphae with intracellular haustoria	Oomycota: *Hyaloperonospora* (O), *Phytophthora* (H), *Albugo* (O, H), *Bremia* (O)
	Basidiomycota (dikaryotic rusts): *Uromyces* (O), *Puccinia* (O)
	Ascomycota (powdery mildews): *Leveillula* (O), *Phyllactinia* (O)
Intracellular hyphae with intracellular haustoria	Basidiomycota (dikaryotic rusts): *Phakopsora* (O), *Physopella* (O)
Initially intracellular hyphae, later intercellular hyphae	Ascomycota: *Magnaporthe* (H), *Colletotrichum* (H), *Claviceps* (F)
	Basidiomycota (monokaryotic rusts): *Uromyces* (O), *Puccinia* (O)
	Basidiomycota (smuts): *Ustilago* (F)
Exclusively intercellular hyphae	Ascomycota: *Cladosporium* (H)
Exclusively intracellular plasmodia	Plasmodiophoromycota: *Plasmodiophora* (F)
	Chytridiomycota: *Olpidium* (F)

O, obligate biotrophy: entirely dependent on the host plant for growth and reproduction, nonculturable; H, hemibiotrophy: initial biotrophic phase followed by necrotrophic phase, culturable; F, faculative biotrophy: ecologically entirely biotrophic but culturable.

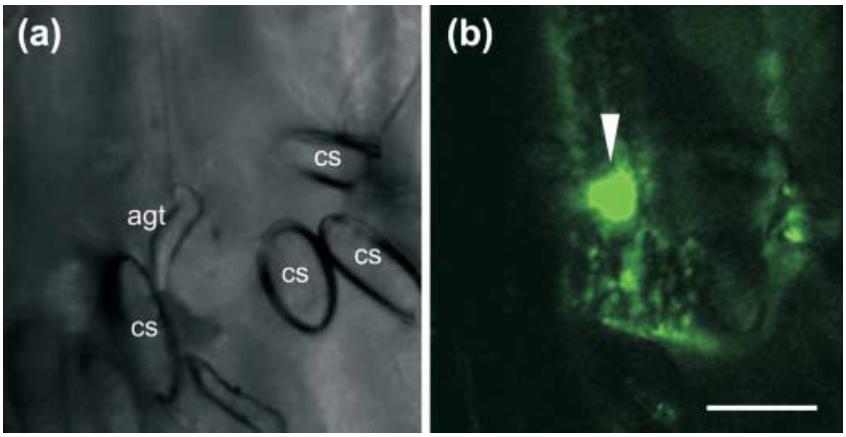

Fig. 1 Focal accumulation of MLO-YFP at an attempted pathogen entry site. The bright field (a) and epifluorescence (b) micrographs represent a section of a barley leaf epidermal cell transiently expressing the MLO-YFP fusion protein (Bhat *et al*., 2005). MLO-YFP focally accumulates at the site of attempted host cell entry (indicated by arrowhead in b) below the appressorial germ tube (agt) of a powdery mildew conidiospore (cs). Bar, 20 μm.

et al., 1997) or chemical signals such as epicuticular waxes (Gniwotta *et al*., 2005 and references therein). Entry into host cells inevitably requires penetration of the plant cell wall and, in the case of epidermal cells, its protective coating of waxes and cutin. Some pathogens, such as *M. grisea* and *Colletotrichum* spp., are thought to breach these barriers using largely physical forces based on appressorial turgor pressure (Howard *et al*., 1991; Bechinger *et al*., 1999), while others, such as the powdery mildews, appear to use a combination of lytic enzymes and turgor pressure (Pryce-Jones *et al*., 1999). On the host side, attempted microbial entry typically leads to major cellular rearrangements, including reorganization of the actin cytoskeleton and organelle movements, which result in a polarization of the host cell towards the site of attack (reviewed in Schmelzer, 2002; Lipka & Panstruga, 2005). Ultimately, host cell polarization usually leads to the formation of local cell wall reinforcements, also termed cell wall appositions or papillae, which are generally believed to function as both physical and chemical barriers to pathogen penetration (Zeyen *et al*., 2002). Two recent studies of plant/powdery mildew interactions demonstrate that not only organelles but also individual host proteins exhibit focal accumulation (local aggregation) at pathogen entry sites in both monocot and dicot plants, thereby defining a plasma membrane microdomain with a unique molecular composition (Fig. 1) (Assaad *et al*., 2004; Bhat *et al*., 2005). In *Arabidopsis thaliana*, the assembly of this microdomain does not appear to be a general wound- or pathogen-associated phenomenon since at least one of its structural components, namely the syntaxin PEN1, does not show focal accumulation at entry sites of the hemibiotrophic fungal pathogen, *Colletotrichum higginsianum* (Shimada *et al*., 2006). This raises the possibility that each pathogen triggers the accumulation of a specific subset of host proteins at sites of attempted ingress. Alternatively, some fungi may suppress the accumulation of some host proteins at attempted entry sites.

Host cell entry is a remarkable biological phenomenon because it entails a significant impairment of plant cell wall integrity without loss of cell viability. Intracellular (hemi-)biotrophs may attempt to limit damage during entry, for example by tightly restricting the extent of enzymic dissolution of host wall polymers (Mendgen *et al*., 1996; Xu & Mendgen, 1997; Herbert *et al*., 2004). However, it is likely that plant cells are able to sense this invasion, for example by associated mechanical wounding or the detection of released plant wall fragments (De Lorenzo *et al*., 2001; Vorwerk *et al*., 2004) and conserved pathogen-derived molecules, the so-called pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). A subset of the latter have been found to act as general elicitors of basal plant immune responses (Nürnberger *et al*., 2004), part of which take place at the cell wall (Hauck *et al*., 2003; Schulze-Lefert, 2004). Local generation of reactive oxygen species (ROS) at the cell periphery, for example, is a frequent plant response to attack by many microbial pathogens. Although the role of ROS in mediating compatibility and/or resistance is controversial (for review, see Hückelhoven & Kogel, 2003), these molecules are generally thought to function in oxidative cell wall cross-linking or plant defence signalling. It appears that (hemi-)biotrophic pathogens have evolved several different mechanisms to cope with this host-derived oxidative stress, including the synthesis and secretion of antioxidative proteins such as catalases and peroxidases (Zhang *et al*., 2004), as well as the generation of metabolites that act as ROS scavengers. For example, the biotrophic infection structures of the bean rust fungus, *Uromyces fabae*, produce mannitol and arabitol which accumulate to high concentrations in the apoplast of infected leaves, where they may function to suppress ROS-related plant defences (Link *et al*., 2005; Voegele *et al*., 2005). Some fungal pathogens may protect themselves from low-molecular-weight antimicrobial compounds (phytoalexins) secreted by attacked plant cells by means of ATP binding cassette (ABC) transporters, which are membrane-localized proton-driven efflux pumps that extrude toxic compounds from the cell. In *M. grisea*, gene-replacement has revealed that the ABC1 transporter is indispensable for colonization of rice and barley epidermal cells (Urban *et al*., 1999), indicating a crucial role for this class of proteins in fungal pathogenesis.

Rare cases of successful cell wall penetration by nonadapted pathogens in so-called nonhost interactions usually trigger hypersensitive cell death of the attacked cells (Lipka *et al.*, 2005). In contrast, host cell wall penetration by compatible pathogens is surprisingly well tolerated, suggesting that, in these instances, host cell entry is accompanied by microbial suppression of host defences and/or cell death (Panstruga, 2003). The failure of nonadapted pathogens to enter cells of a given plant species successfully may therefore result from their inability to cope with the 'bouquet' of possibly species-specific defences that greets them at the plant cell periphery.

2. PENs and co: gatekeepers at the cell periphery

Which are the plant molecules that limit microbial ingress by nonadapted pathogens? A range of genetic studies recently shed light on host genes involved in restricting the entry of the nonadapted barley powdery mildew, *Blumeria graminis* f.sp. *hordei*, into Arabidopsis leaf epidermal cells. Usually, most attempts at cell wall penetration by fungal sporelings fail in this 'nonhost' pathosystem. In contrast, Arabidopsis mutants defective at any of three distinct *PENETRATION* (*PEN*) loci, encoding a syntaxin (PEN1), a glycosyl hydrolase (PEN2), and an ABC transporter (PEN3), respectively, exhibit elevated rates of fungal host cell entry (Collins *et al.*, 2003; Lipka *et al.*, 2005; Stein *et al.*, 2006). Syntaxins are members of the superfamily of SNARE domain-containing proteins that are known to mediate membrane fusion events during exo- and endocytosis in yeast and animal cells (Bonifacino & Glick, 2004). The contribution of a syntaxin in resistance to nonadapted pathogens has thus been interpreted as evidence for a key role in vesicle trafficking and polarized secretion during basal resistance (Schulze-Lefert, 2004). This hypothesis is further supported by the fact that another Arabidopsis syntaxin isoform, AtSYP122, is subject to rapid phosphorylation upon treatment with the general bacterial elicitor, flagellin (Nühse *et al.*, 2003), while yet another syntaxin isoform, AtSYP132, has been recently implicated in race-specific resistance of Arabidopsis to a bacterial pathogen (Heese *et al.*, 2005). In addition, a further SNARE domain protein, the barley SNAP25 homolog HvSNAP34, has been shown in yeast two-hybrid assays to interact with the barley ROR2 syntaxin (Collins *et al.*, 2003). Based on gene silencing experiments, it has been demonstrated that HvSNAP34 contributes to basal defence against adapted and nonadapted powdery mildew species (Collins *et al.*, 2003; Douchkov *et al.*, 2005). Finally, recent gene expression analysis uncovered coordinated transcriptional up-regulation of genes encoding components of the secretory pathway during the systemic acquired resistance response in *A. thaliana* (Wang *et al.*, 2005), a plant-wide type of broad-spectrum immunity that is triggered upon local contact with an inducing pathogen. In conclusion, secretion emerges as a key molecular process in various types of plant defence and it is evident that adapted pathogens must have evolved means to cope with this challenge in order to establish compatibility. It remains a major goal for the future to unravel the molecules – polypeptides and/or low-molecular-weight compounds – that are released via the plant secretory pathway at attempted entry sites. It is likely that antimicrobial peptides and toxic secondary metabolites such as phytoalexins will comprise at least part of this cargo.

PEN2 encodes a peroxisome-associated family 1 glycosyl hydrolase (Lipka *et al.*, 2005). Family 1 glycosyl hydrolases are known to catalyse the hydrolysis of O- or S-glycosidic bonds between two or more carbohydrates or between a carbohydrate and a noncarbohydrate (also referred to as an aglycone). Contribution of this activity to nonhost resistance in combination with the observed focal accumulation of PEN2-associated peroxisomes at fungal entry sites suggests that cleavage of the glycosidic bond of a possibly peroxisome-derived substrate might result in local release of one or more compounds with antifungal activity (Lipka *et al.*, 2005). Mutations in the ABC transporter PEN3 (also known as PDR8) surprisingly confer opposite phenotypes to adapted and nonadapted powdery mildews: while *pen3* mutants allow enhanced entry by the nonadapted host grass powdery mildew, *B. graminis*, they mediate partial postinvasion resistance to the adapted powdery mildew species, *Golovinomyces* (formerly *Erysiphe*) *cichoracearum* (Stein *et al.*, 2006).

3. Belt-and-braces immunity: postinvasion defences provide the backup parachute

Although mutations in each of the three *PEN* genes suffice to compromise defence pathway(s) that restrict fungal entry into host cells, none of the *pen* mutants supports substantial surface hyphal growth following plant cell invasion. In contrast, successful ingress is in most cases accompanied by host cell death, thereby impeding further expansion of fungal microcolonies (Lipka *et al.*, 2005). This finding led to the conclusion that at least one layer of postinvasion defence may exist that operates independently of host entry control at the cell periphery. Indeed, double and triple mutant analysis involving the *pen2* mutant revealed that *PAD4* and *SAG101*, two genes previously implicated in resistance (*R*) gene-mediated immunity and basal defence, are key players in postinvasive nonhost immunity. Lipase-like proteins PAD4 and SAG101 are thought to amplify salicylic acid-dependent defence signalling, possibly via the formation of hetero-oligomeric complexes and nucleocytoplasmic shuttling involving a further lipase-like polypeptide, EDS1 (Feys *et al.*, 2005). Triple mutants *pen2 pad4 sag101* allow substantial fungal penetration and support extensive surface hyphal growth and even sporulation (conidiophore formation) by nonadapted powdery mildew fungi (Lipka *et al.*, 2005). Since completion of the asexual life cycle is the hallmark of compatibility, mutations in these three genes turn a previously incompatible plant–pathogen interaction into a truly compatible one. In conclusion, it seems that, at least in the dicotyledonous reference plant *A. thaliana*, adapted powdery

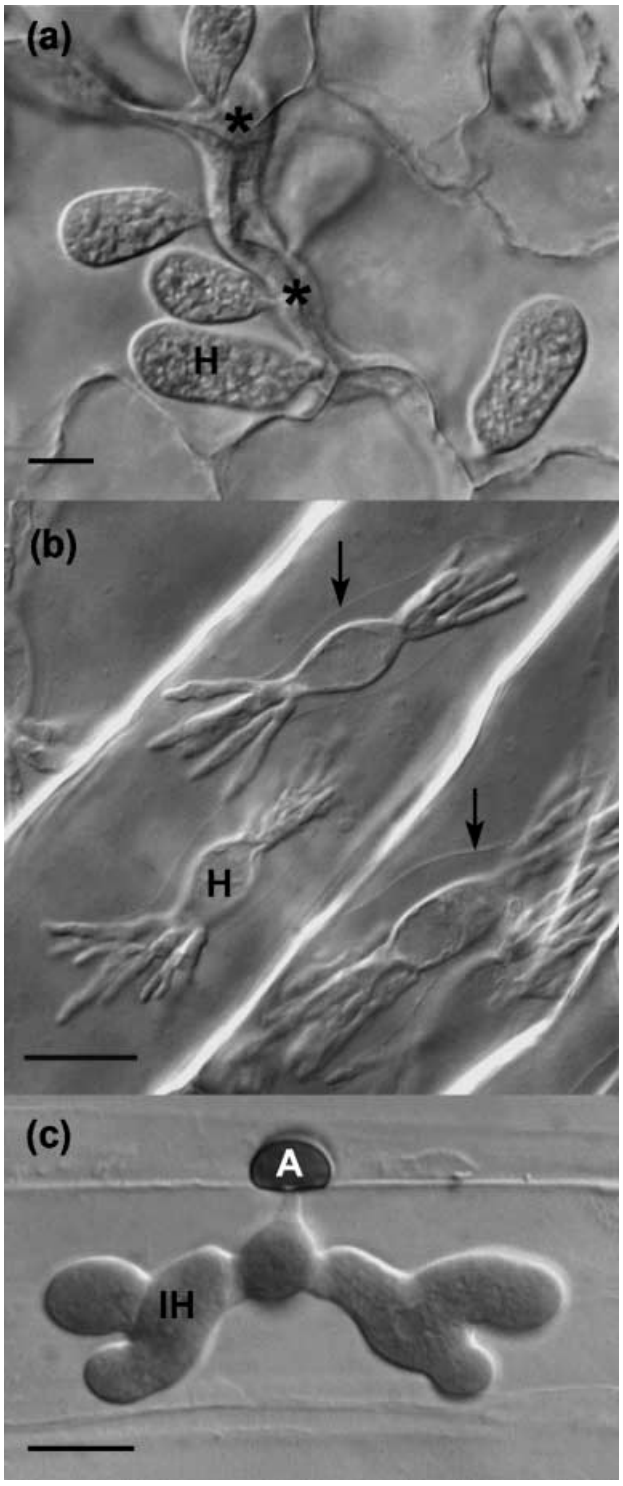

Fig. 2 Light micrographs illustrating the infection structures of some intracellular biotrophs. (a) Haustoria (H) developing from intercellular hyphae (*) of the obligately biotrophic oomycete *Hyaloperonospora parasitica* inside epidermal cells of *Brassica oleracea*. (Image provided by Raffaella Carzaniga, Rothamsted Research, Hertfordshire, UK.) (b) Haustoria (H) of the obligately biotrophic powdery mildew fungus *Blumeria graminis* f.sp. *avenae* developing inside epidermal cells of *Avena fatua*. Arrows indicate the extrahaustorial membrane. (Image provided by George Barron from the MycoAlbum CD-ROM, University of Guelph, Guelph, Ontario, Canada.) (c) Intracellular hyphae of the hemibiotrophic crucifer anthracnose fungus *Colletotrichum higginsianum* have developed from a melanized appressorium (A) and penetrated into an epidermal cell of *Arabidopsis thaliana*. Bars, 10 μm.

mildews are able to overcome two separate defensive layers: first, preinvasive protection at the cell periphery, which restricts microbial entry; and second, salicylic acid-dependent postinvasive cytoplasmic defences, frequently resulting in hypersensitive cell death, that limit subsequent parasite proliferation and sporulation. This multilayered defence system and the complex, multifactorial nature of each component may explain why nonhost resistance is the most durable form of plant immunity in nature (Nürnberger & Lipka, 2005).

III. The plant–biotroph interface

1. Haustoria and intracellular hyphae: beachheads for feeding and host reprogramming

Having successfully evaded host defences associated with host cell entry, (hemi-)biotrophs can elaborate their specialized intracellular infection structures. Haustoria develop as side branches from intercellular, intracellular and epicuticular hyphae and terminate inside the penetrated host cell (Figs 2a,b, 3a). They are the hallmark of all obligate biotrophs, including powdery mildews, rusts and oomycetes (Voegele & Mendgen, 2003). By contrast, filamentous intracellular hyphae can penetrate from cell to cell, colonizing a small number of host cells, and are produced by both obligate biotrophs, for example monokaryotic rusts (Gold & Mendgen, 1984), and hemibiotrophs, such as species of *Magnaporthe* and *Colletotrichum* (O'Connell *et al.*, 1985; Heath *et al.*, 1992; Wharton *et al.*, 2001). The intracellular hyphae of *Colletotrichum destructivum* and *C. higginsianum* are an interesting exception because, like haustoria, they are restricted to a single host cell (Fig. 2c), although the necrotrophic hyphae that later develop from them invade many cells (Latunde-Dada *et al.*, 1996; Shen *et al.*, 2001; O'Connell *et al.*, 2004). Following penetration of the plant cell wall, both intracellular hyphae and haustoria develop inside the cell lumen but they always remain outside the plant plasma membrane. The interface formed between the two organisms typically comprises the plasma membrane and cell wall of the biotroph, a plant-derived interfacial membrane (the extrahaustorial membrane of haustoria) and an interfacial matrix layer (the extrahaustorial matrix of haustoria) which separates the interfacial membrane from the pathogen cell wall (Fig. 3b). The plant–biotroph interface is believed to function as a key 'trading place' for the uptake of nutrients into the pathogen and export of pathogen effector molecules into host cells (Voegele & Mendgen, 2003). Here we review what is currently known about the unique properties of this interfacial zone.

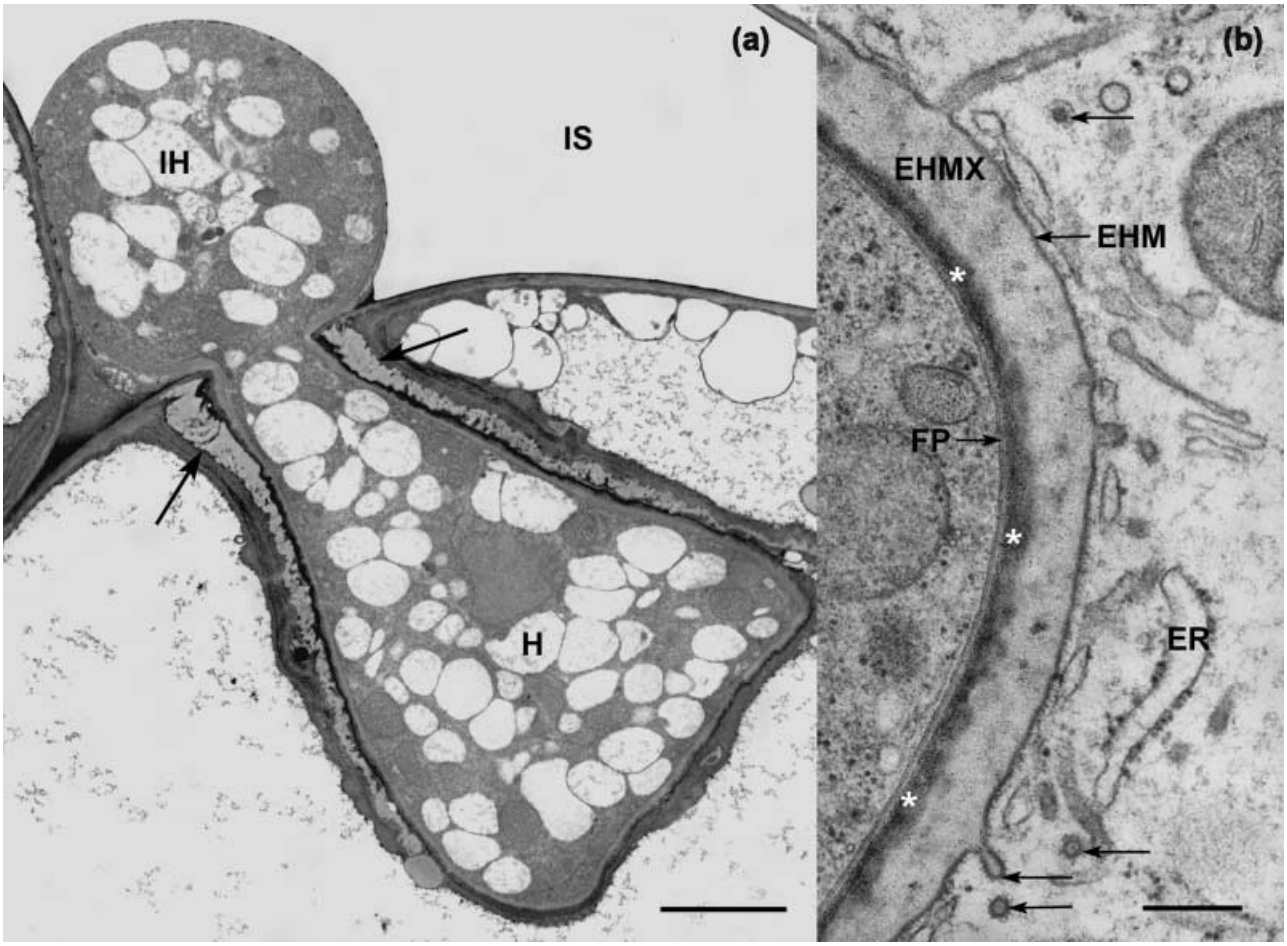

Fig. 3 Transmission electron micrographs illustrating the structure of interfaces developed with plant cells by haustoria and intracellular hyphae. (a) Compatible interaction between the oomycete *Hyaloperonospora parasitica* and *Brassica oleracea*. An intercellular hypha (IH) developing in the intercellular space (IS) has produced a haustorium (H) inside a mesophyll cell. Note that the haustorium is partially encased by a layer of callose (arrows) deposited between the extrahaustorial membrane and the extrahaustorial matrix. Bar, 5 μm. (b) The interface between *Vigna sinensis* and the growing tip of an intracellular hypha of the monokaryotic rust *Uromyces vignae*. The interface comprises the fungal plasma membrane (FP), fungal cell wall (*), extrahaustorial matrix (EHMX) and extrahaustorial membrane (EHM). Tubular coated pits on the EHM and coated vesicles in the surrounding cytoplasm (arrows) indicate that plant endocytosis occurs at the interface. ER, endoplasmic reticulum. Bar, 1 μm. (Image provided by Martina Stark-Urnau and Kurt Mendgen, University of Konstanz, Konstanz, Germany.)

2. Biotroph plasma membranes: elaborate nutrient suckers

The development of procedures to purify the haustoria of rusts and powdery mildews from infected plant tissue (Gil & Gay, 1977; Mackie *et al.*, 1991; Hahn & Mendgen, 1992) was a major technical advance that made possible the molecular analysis of haustoria, including the identification of components specific to haustorial plasma membranes. For example, monoclonal antibodies raised to isolated haustorial complexes of the pea powdery mildew, *Erysiphe pisi*, identified two glycoproteins of unknown function that are present only in haustorial plasma membranes and not the plasma membranes of epicuticular hyphae (Mackie *et al.*, 1993). A cDNA library prepared from isolated haustoria of the bean rust fungus, *U. fabae*, has been an extraordinarily rich source of *in planta*-induced fungal genes (PIGs) (Hahn & Mendgen, 1997; Jakupovic *et al.*, 2006). One of these genes, *HXT1*, encodes a hexose transporter that is highly expressed in haustoria and exclusively localized in the haustorial plasma membrane, where it likely mediates the uptake of D-glucose and D-fructose from the extrahaustorial matrix (Voegele *et al.*, 2001). This important study provided the first proof that haustoria are engaged in sugar uptake and that this activity may be restricted to haustoria. Since the concentration of hexoses in the plant apoplast is low, it was proposed that substrates for HXT1 derive from the cleavage of sucrose by invertase enzymes. Consistent with this hypothesis, the rust invertase gene *Uf-INV1* was recently found to be highly expressed in haustoria, with the enzyme protein being secreted into the extrahaustorial matrix (Voegele *et al.*, 2006).

However, expression of a *Vicia faba* cell wall-associated invertase, *CWINV2*, also increased in rust-infected leaves and the possibility that this host enzyme generates additional hexose at the plant–fungal interface cannot be excluded.

Three other bean rust PIGs encode plasma membrane amino acid transporters that are highly expressed in haustoria but also in intercellular hyphae and earlier infection structures (Hahn *et al.*, 1997; Struck *et al.*, 2002), indicating that amino acid uptake is not the exclusive role of haustoria (Voegele & Mendgen, 2003). The translocation of monosaccharides and amino acids by these symporters must be coupled to the generation of an electrochemical gradient across the haustorial plasma membrane by a proton pump. The rust plasma membrane H^+-ATPase, *Uf-PMA1*, has also been cloned and characterized biochemically in yeast, but although elevated enzyme activity was detected in isolated haustorial plasma membranes (Struck *et al.*, 1996), transcript abundance was actually higher in some other fungal structures, suggesting that *Uf-PMA1* expression is subject to post-transcriptional regulation (Struck *et al.*, 1998). Using quantitative PCR, Both *et al.* (2005b) found that the putative plasma membrane H^+-ATPase of *B. graminis* f.sp. *hordei* was up-regulated in infected barley epidermal strips, supporting a similar role for this enzyme in nutrient acquisition by powdery mildew haustoria. In both *B. graminis* and *U. fabae*, transcript profiling using cDNA microarrays indicates that wholesale changes in fungal gene expression occur during the switch from preinfection development to biotrophic growth, including the co-ordinate regulation of entire suites of genes encoding enzymes in similar pathways of primary metabolism (Both *et al.*, 2005a; Jakupovic *et al.*, 2006).

Haustoria and intracellular hyphae occupy a similar niche within plant cells, and although they generally develop a less specialized interface than haustoria, intracellular hyphae may share similar functions. Immunolocalization of HXT1p and the amino acid transporter AAT2p in the apices of intracellular hyphae formed during the monokaryotic phase of *U. fabae* supports the view that they function as feeding structures (Mendgen *et al.*, 2000; Voegele & Mendgen, 2003). However, in the case of hemibiotrophs there is currently no evidence to show whether intracellular hyphae (e.g. those formed by *M. grisea* and species of *Colletotrichum*) or haustoria (e.g. those formed by species of *Phytophthora*) play any role in nutrient uptake.

3. Biotroph cell walls: PAMP-packed pathogen identity cards

Little information is available on the wall composition of intracellular infection structures, but there is evidence that some biotrophs and hemibiotrophs modify their cell walls during growth inside plant cells. For example, monoclonal antibodies raised to isolated haustoria of the flax rust, *Melampsora lini*, recognized three oligosaccharide epitopes only present in haustorial cell walls and not other fungal cell types (Murdoch & Hardham, 1998). Structural polysaccharides in the walls of fungi and oomycetes such as chitin and β-1,3-glucans are PAMPs, which can be recognized by cell surface receptors to activate basal plant defence responses (Nürnberger *et al.*, 2004). During invasion of plant tissue, these polymers may also be susceptible to attack by chitinases and β-1,3-glucanases present in the apoplast and the plant–fungal interface, potentially causing the lysis of hyphal tips (Mauch & Staehelin, 1989; Hu & Rijkenberg, 1998). It may therefore be advantageous for biotrophs to limit their exposure of these polymers during growth *in planta*. Lectin cytochemistry has shown that chitin is indeed absent or inaccessible in the walls of haustorial necks and young rust haustoria (Harder & Chong, 1984) and in the penetration pegs and young intracellular hyphae of *Colletotrichum lindemuthianum* (O'Connell & Ride, 1990), where these pathogens first come into close contact with the host plasma membrane. One plausible explanation is that developmentally regulated chitin deacetylase enzymes convert the chitin into chitosan, which may allow these fungi to evade lysis by plant chitinases at a crucial early stage of intracellular growth (Siegrist & Kauss, 1990; Deising & Siegrist, 1995; El Gueddari *et al.*, 2002). However, the deacetylation of cell wall chitin may not circumvent plant recognition because chitosan is also a PAMP (Agrawal *et al.*, 2002). In powdery mildews and oomycetes, cell wall chitin and β-1,3 glucans are detectable at all stages of haustorial development (Enkerli *et al.*, 1997; Mims *et al.*, 2004; Ramonell *et al.*, 2005). Presumably these biotrophic pathogens have evolved mechanisms either to avoid or to suppress plant perception of these PAMPs at the interface with their hosts.

4. The matrix reloaded: a mélange of plant and pathogen components

An interfacial matrix surrounds the intracellular infection structures of nearly all (hemi-)biotrophic pathogens and mycorrhiza fungi, with the possible exception of some hemibiotrophs (Heath *et al.*, 1992; Wharton *et al.*, 2001; O'Connell *et al.*, 2004). It is currently impossible to isolate interfacial matrices for direct biochemical analysis, so most information on their composition has come from the use of antibodies, lectins and enzymes as affinity probes for *in situ* cytochemistry. In the case of rust haustoria, many polysaccharides and glycoproteins typical of primary plant cell walls have been detected in the extrahaustorial matrix, including pectins, xyloglucan, arabinogalactan proteins, hydroxyproline-rich glycoprotein and threonine-hydroxyproline-rich glycoprotein (Stark-Urnau & Mendgen, 1995; Hippe-Sanwald *et al.*, 1994). In striking contrast, plant cell wall components are generally not detectable in extrahaustorial matrices of powdery mildews (Hajlaoui *et al.*, 1991; Green *et al.*, 1995; Celio *et al.*, 2004). Perhaps because organized wall components are lacking, the extrahaustorial matrices of powdery mildews generally appear liquid or gel-like in consistency (Manners & Gay, 1983).

The failure to detect plant wall components in powdery mildew extrahaustorial matrices has led to the suggestion that either the pathogen suppresses their synthesis and secretion at the interface or they become degraded by fungal hydrolytic enzymes after secretion into the matrix, perhaps providing a source of nutrition to the pathogen (Green *et al.*, 2002). A further possibility is that plant lytic enzymes and wall-loosening proteins prevent secreted matrix components from assembling into a normal, cross-linked cell wall (Balestrini & Bonfante, 2005). Support for this notion comes from the analysis of plant gene expression in mycorrhizal roots, which has shown that several plant genes implicated in wall remodelling are up-regulated in cells containing arbuscules, for example expansin, xyloglucan endotransglucosylase, alpha-fucosidase and a membrane-anchored endo-1,4-β-glucanase (Liu *et al.*, 2003, 2004; Maldonado-Mendoza *et al.*, 2005). If intracellular biotrophic pathogens exploit the same 'accommodation' pathway as endosymbionts to enter plant cells, as proposed by Parniske (2000), perhaps they induce similar host genes during infection. In this context, it is interesting to note that a mutant screen in Arabidopsis identified a GPI-anchored pectate lyase-like protein, PMR6, as a plant factor essential for susceptibility to powdery mildews (Vogel *et al.*, 2002; also discussed later). One possibility is that PMR6 is attached to the outer surface of the extrahaustorial membrane and modifies pectins within the extrahaustorial matrix, which may be essential for proper functioning of the powdery mildew haustorium.

Interfacial matrices generally do not contain fungal wall polysaccharides such as chitin, but immunolabelling has demonstrated the presence of fungal proteins in the extrahaustorial matrix. For example, a glycoprotein elicitor was detected in the extrahaustorial matrix of the cereal stem rust, *Puccinia graminis tritici* (Marticke *et al.*, 1998), while haustoria of the bean rust *U. fabae* secrete rust transferred protein 1 (*Uf*-RTP1p) and invertase *Uf*-INV1p into the extrahaustorial matrix (Kemen *et al.*, 2005; Voegele *et al.*, 2006; also discussed later). The intracellular hyphae of *C. lindemuthianum* secrete a 45 kDa proline-rich glycoprotein, CIH1p (Colletotrichum intracellular hypha 1), into the interfacial matrix, where it appears to become oxidatively cross-linked (Perfect *et al.*, 1998, 2000). Although the expression of *CIH1* is tightly linked to the intracellular biotrophic phase, its function remains unclear. Further evidence supporting a role for the fungal partner in interface development comes from the maize smut fungus, *Ustilago maydis*. In mutants defective in an α-glucosidase (*gas1*), which processes *N*-linked glycoproteins in the endoplasmic reticulum (ER), the biotrophic intracellular hyphae of this fungus failed to develop a normal interfacial matrix and their growth became arrested within the epidermis, without the expression of host defence responses (Schirawski *et al.*, 2005). This suggests that proper glycosylation of fungal cell wall or matrix proteins is essential for establishment of a functional biotrophic interface in this pathosystem.

5. Separating the wheat from the chaff: interfacial membranes as molecular sieves

There is abundant evidence that obligate biotrophs induce the formation of a highly modified interfacial membrane that is markedly different in structure and composition from the plasma membrane lining the plant cell wall. An early transmission electron microscopy (TEM) observation, later confirmed using modern cryofixation techniques, was that the extrahaustorial membranes of rusts and powdery mildews appear smooth after freeze-fracture, lacking the punctate intramembrane particles that are typical of normal membranes (Knauf *et al.*, 1989). Since these represent the cross-fractured transmembrane domains of integral membrane proteins (Eskandari *et al.*, 1998), these findings suggest that the extrahaustorial membrane is highly depleted in such proteins. The extrahaustorial membranes of powdery mildews are also thicker than the normal plasma membrane, as a result of associated carbohydrate material, are more resistant to detergents and osmotic shock, and are highly corrugated near the haustorial neck, with complex folds and tubules extending into the extrahaustorial matrix (Manners & Gay, 1983; Mims *et al.*, 2003).

The lipid composition of extrahaustorial membranes has not been analysed but one consistent feature is that, unlike normal plasma membranes, they stain poorly with phosphotungstic acid, which is thought to label membrane glycolipids (Soylu, 2004 and references therein). Recently the polyene antibiotic filipin has gained popularity as a fluorescent marker for sterol-enriched plasma membrane microdomains, termed lipid-rafts (Bhat & Panstruga, 2005). In a much earlier study, freeze-fracture TEM following filipin treatment revealed an absence of granular filipin-sterol complexes on the extrahaustorial membranes of two rust fungi, *Puccinia coronata* and *Uromyces appendiculatus* (Harder & Mendgen, 1982). This suggests that the extrahaustorial membrane contains less sterol than normal plasma membranes, which could affect both its fluidity and permeability.

To date, the only known example of an extrahaustorial membrane-specific protein is a 250 kDa glycoprotein identified in the extrahaustorial membrane of *E. pisi* using a monoclonal antibody raised to isolated haustorial complexes (Roberts *et al.*, 1993). The glycoprotein is present from the earliest stages of haustorium formation but is not detectable in older haustoria, showing that the composition of the extrahaustorial membrane changes during development. The corresponding gene has not been cloned and, although probably of plant origin, it remains possible that the protein is secreted by the fungus and then becomes inserted into the extrahaustorial membrane. The only other example of a protein that is targeted specifically to an interfacial membrane comes from mycorrhizal interactions in *Medicago truncatula* roots, where the plant phosphate transporter MtPT4 localizes exclusively to the periarbuscular membrane and may function in phosphate uptake from the endosymbiont into the plant (Harrison *et al.*, 2002). A major challenge now is to discover more proteins that are unique to

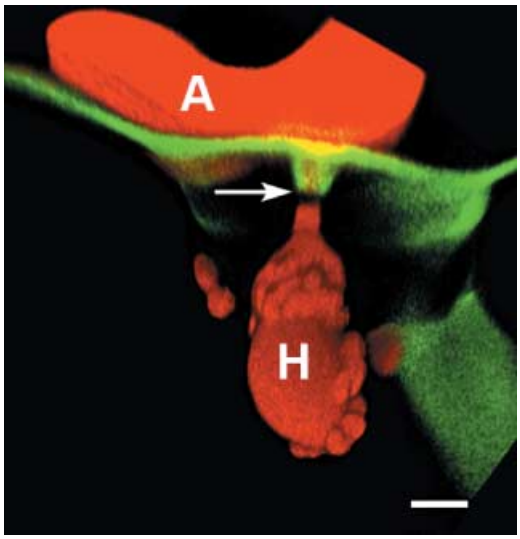

Fig. 4 A live-cell confocal microscope image illustrating differentiation of the extrahaustorial membrane (EHM) around a haustorium (H) of *Erysiphe cichoracearum* in an epidermal cell of *Arabidopsis thaliana*. A green fluorescent protein (GFP)-tagged plasma membrane marker, shown in green, is present in the wall-lining plasma membrane but is excluded from the EHM around the haustorial body, with an abrupt transition in membrane labelling at the haustorial neck (arrow). Fungal structures are stained red with propidium iodide. This three-dimensional volume-rendered image was generated from a Z-series of 19 optical sections (0.5 μm thick). A, appressorium. Bar, 4.5 μm. (Reproduced from Koh *et al*. (2005), with permission from Blackwell Publishing Ltd.)

biotroph interfacial membranes and the mechanisms that specifically target them to this membrane.

Although some highly glycosylated plant plasma membrane glycoproteins, including arabinogalactan proteins, may be present in the extrahaustorial membrane (Green *et al.*, 1995), there is growing evidence that many other plasma membrane-resident proteins are excluded. For example, in the *E. pisi*–pea interaction, the plant plasma membrane glycoprotein recognized by monoclonal antibody UB9 is absent from the extrahaustorial membrane around young haustoria, although it can be detected in a subset of older haustoria (Roberts *et al.*, 1993). More recently, Koh *et al.* (2005) used live-cell confocal imaging to show that in Arabidopsis epidermal cells infected by *E. cichoracearum*, eight different green fluorescent protein (GFP)-tagged plasma membrane marker proteins are all excluded from the extrahaustorial membrane, including three aquaporins, a syntaxin and a brassinosteroid receptor (Fig. 4). There is also evidence that the ATPase activity normally associated with plant plasma membranes is absent from the extrahaustorial membranes around obligate biotrophs, which could favour a unidirectional flow of nutrients towards the pathogen (Woods *et al.*, 1988; Smith & Smith, 1990; Baka *et al.*, 1995). However, the specificity of the cytochemical technique used in these studies is questionable and the absence of a plasma membrane H^+-ATPase from the extrahaustorial membrane requires verification by immunocytochemistry or localization of a GFP-tagged H^+-ATPase in living cells (Lefebvre *et al.*, 2004).

For most intracellular biotrophs, there is unequivocal TEM evidence that the highly differentiated extrahaustorial membrane is continuous with the normal plasma membrane lining the plant cell wall. In haustoria of dikaryotic rusts and powdery mildews, the transition in membrane properties is abrupt and occurs at the haustorial neck, associated with one or two annular structures called neckbands which appear to maintain separation between the two membrane domains (Heath & Skalamera, 1997, Fig. 4). In addition, neckbands fuse both the haustorial plasma membrane and extrahaustorial membrane on to the neck wall and effectively block the diffusion of solutes along the neck wall (Manners & Gay, 1983). This creates a separate apoplastic compartment, comprising the haustorial wall and extrahaustorial matrix, an arrangement that may facilitate not only nutrient uptake by the pathogen but also the targeted export of pathogen effectors directly into host cells (described later). The haustoria of most biotrophic oomycetes and the intracellular hyphae of monokaryotic rusts, *Magnaporthe* and *Colletotrichum* lack any detectable neckband and perhaps, as a consequence, develop less specialized interfacial membranes (Woods & Gay, 1983; O'Connell, 1987). However, a recent study using live-cell confocal imaging suggests that the intracellular hyphae of *C. higginsianum* can modify the host membrane because a GFP-tagged plasma membrane-resident syntaxin, AtPEN1, was excluded from the membrane surrounding mature intracellular hyphae (Shimada *et al.*, 2006).

6. Snuggling up: accommodation of biotrophic infection structures by plant cells

Compatibility between plants and intracellular biotrophs requires plant cells to accommodate relatively large pathogen infection structures without loss of cell viability. A key aspect of this process must be the precisely regulated expansion and differentiation of the plant plasma membrane to form a new extrahaustorial membrane. This presumably involves a pathogen-induced redirection of the plant secretory pathway, and indeed both plant ER and Golgi stacks are frequently reported to proliferate around the haustoria of obligate biotrophs (Leckie *et al.*, 1995; Heath & Skalamera, 1997; Takemoto *et al.*, 2003; Koh *et al.*, 2005). Similarly, GFP tagging shows that the plant cortical ER (but not Golgi) accumulates around intracellular hyphae of *C. higginsianum* and undergoes a local reorganization from a loose tubular arrangement to a compact cisternal format (R. O'Connell, unpublished), which may reflect increased synthesis of host proteins and lipids (Ridge *et al.*, 1999).

Two alternative models for biogenesis of the extrahaustorial membrane were recently proposed by Koh *et al.* (2005). In one scenario, invagination and stretching of the host plasma membrane by the growing haustorium is compensated by the addition of new membrane material by exocytosis all around the cell periphery, with the neckband acting as a 'sieve' to allow certain

membrane components to enter the extrahaustorial membrane while excluding others. The second model envisages a completely *de novo* synthesis of extrahaustorial membrane by the targeted secretion of novel extrahaustorial membrane-specific vesicles, whose membranes presumably lack normal plasma membrane-resident proteins. The latter hypothesis could also account for simultaneous biogenesis of the extrahaustorial matrix.

At sites of rapid plasma membrane expansion in plant cells, for example cell plates and the tips of pollen tubes and root hairs, the exocytosis of new membrane and wall material is always coupled with membrane recycling by endocytosis (Hepler *et al.*, 2001). There is very little convincing TEM evidence that plant endocytosis occurs at biotrophic interfaces (O'Connell, 1987; Xu & Mendgen, 1994; Bauer *et al.*, 1995). However, the interfacial membrane around growing tips of the monokaryotic intracellular hyphae of *U. vignae* does contain tubular clathrin-coated pits that could mediate plant endocytosis (Stark-Urnau & Mendgen, 1995; Fig. 3b). In addition to membrane recycling, this may provide a route by which components of the interfacial matrix, perhaps including pathogen effectors, could be internalized into host cells.

Surprisingly, Koh *et al.* (2005) found evidence in the Arabidopsis–powdery mildew interaction that pouches of excess plasma membrane accumulate at the penetration site before fungal entry, into which the haustorium subsequently grows. This suggests that expansion of the plant membrane may not be tightly linked to growth of the haustorium and that fungal signals prepare the cell for invasion. Further evidence that the accomodation pathway may be induced in plant cells before fungal entry was recently obtained in a symbiotic interaction (Genre *et al.*, 2005). Confocal imaging of GFP-tagged plants showed that long tubular structures composed of host ER, microtubules and actin filaments are assembled by root epidermal cells before penetration by hyphae of a mycorrhiza fungus, and this 'prepenetration apparatus' was proposed to play a role in interface biogenesis.

IV. Biotroph effectors

1. Pathogen effectors: the hunt is on!

It is believed that pathogenic microbes generally synthesize and release effector proteins for targeted manipulation of their respective host species. In the case of phytopathogenic bacteria, the delivery route for these effectors, the so-called type three secretion system, is well known. It represents a kind of molecular syringe that is docked on to host cells and through which the effector polypeptides are funnelled into the plant cytoplasm (Jin *et al.*, 2003). Genome analysis revealed that the hemi-biotrophic bacterial phytopathogen, *Pseudomonas syringae*, encodes more than 40 distinct effectors (Alfano & Collmer, 2004). In Arabidopsis a few of these are recognized by matching resistance (*R*) genes present in certain ecotypes, and are therefore considered to be avirulence (*Avr*) factors. It appears that the primary task of a subset of these effector polypeptides is related to suppressing host defences and/or programmed cell death (reviewed in Alfano & Collmer, 2004; Chang *et al.*, 2004). At the biochemical level, the functions of bacterial effector proteins have been shown to include protease, kinase, phosphatase and pectate lyase activities (Alfano & Collmer, 2004; Chang *et al.*, 2004). Since the primary amino acid sequence in many cases does not provide any clue to their biochemical function, the exact role of many bacterial effectors during pathogenesis remains unknown. In these instances, structural analysis might be required to uncover possible functions, as recently demonstrated for AvrPtoB, which was found to mimic the structure of a eukaryotic E3 ubiquitin ligase (Janjusevic *et al.*, 2006).

In contrast to bacteria, little is known about the identity, release, uptake, and function of the effectors of (hemi-)biotrophic fungal and oomycete pathogens. Previously, many proteins secreted by these pathogens have been isolated and characterized biochemically. This led to the discovery of various polypeptides that primarily localize to the plant apoplast, many of which are enzyme inhibitors or proteases which probably function in microbial counterdefence to secreted plant pathogenesis-related proteins such as chitinases and endoglucanases (reviewed in Kamoun, 2006). A well-known example of this class of effectors is the glucanase inhibitor protein 1 (GIP1) of *Phytophthora sojae* which differentially targets β-1,3-endoglucanases of various *Glycine* species (Ham *et al.*, 1997; Bishop *et al.*, 2005). In addition to these effectors that act in the plant apoplast, there is increasing evidence that a substantial portion of the effector proteins secreted by (hemi-)biotrophic pathogens act inside the host cell, for example in the cytoplasm or in the nucleus. In many cases this has been indirectly inferred from the fact that the effectors are recognized as avirulence (Avr) determinants by matching resistance (R) proteins with either proven or assumed cytoplasmic localization. The presence of matching Avr–R protein pairs usually results in a typical hypersensitive cell death response. A cytoplasmic localization has also been deduced from the observation that expression of some pathogen effectors inside plant cells triggers cell death, suggesting intracellular recognition of the respective effector by as yet unknown R proteins or other types of pattern recognition receptors (Torto *et al.*, 2003; Table 2).

In many cases where pathogen effectors are presumed to act intracellularly, they appear to be synthesized in haustoria/intracellular hyphae, channelled through the pathogen's secretory pathway and finally discharged into the interfacial matrix via exocytosis (Kemen *et al.*, 2005; Catanzariti *et al.*, 2006; Ellis *et al.*, 2006). This view is supported by the fact that: (i) transcripts encoding certain effectors are enriched in haustoria (Catanzariti *et al.*, 2006); and (ii) many, though not all, of the identified intracellular effectors carry prototypical N-terminal secretion signals (to be described later, Table 2). How these polypeptides are subsequently taken up from the interfacial space into the plant cell remains a mystery, however (Ellis *et al.*, 2006). Possibly a first step towards resolving this puzzle

Table 2 A selection of effector proteins from (hemi-)biotrophic plant pathogens predicted to act inside host cells

Effector protein	Parasite species	Host species	Predicted N-terminal signal peptide	Evidence for localization inside the host cell	Evidence for diversifying selection	Reference
ATR1	*Hyaloperonospora parasitica*	*Arabidopsis thaliana*	+	+[a]	+	Rehmany *et al*. (2005)
ATR13	*Hyaloperonospora parasitica*	*Arabidopsis thaliana*	+	+[a]	+	Allen *et al*. (2004)
Avr1b-1	*Phytophthora sojae*	*Glycine max*	+	+[b]	+	Shan *et al*. (2004)
Avr3a	*Phytophthora infestans*	*Solanum tuberosum*, *Lycopersicon esculentum*	+	+[a]	+	Armstrong *et al*. (2005)
Avr_{a10}	*Blumeria graminis*	*Hordeum vulgare*	–	+[b]	nt	Ridout (2004)
Avr_{k1}	*Blumeria graminis*	*Hordeum vulgare*	–	+[c]	nt	Ridout (2004)
AvrL5, AvrL6, AvrL7	*Melampsora lini*	*Lycopersicon usitatissimum*	+	+[a,d]	+	Dodds *et al*. (2004)
AvrM	*Melampsora lini*	*Linum usitatissimum*	+	+[a,d]	+	Catanzariti *et al*. (2006)
AvrP4	*Melampsora lini*	*Linum usitatissimum*	+	+[a,d]	+	Catanzariti *et al*. (2006)
Avr Pi-ta	*Magnaporthe grisea*	*Oryza sativa*	+	+[a]	nt	Jia *et al*. (2000)
CRN1, CRN2	*Phytophthora infestans*	*Solanum tuberosum*, *Lycopersicon esculentum*	+	+[d]	nt	Torto *et al*. (2003)
RTP1	*Uromyces fabae*	*Vicia faba*	+	+[e]	nt	Kemen *et al*. (2005)
	Uromyces striatus	*Medicago truncatula*	+	+[e]	nt	

nt, not tested (because of lack of multiple available sequences).
Inferred from [a]the predicted cytoplasmic localization of the matching R protein,
[b]the predicted cytoplasmic localization of an allele of the matching R protein,
[c]the proven cytoplasmic localization of the matching R protein of the sequence-related Avr_{a10} effector,
[d]induced cell death upon cytoplasmic expression,
[e]immunolocalization experiments.

is the recent identification of a conserved amino acid sequence motif, RXLR (R, arginine; L, leucine; X, any amino acid), that is present in close spatial proximity to the predicted signal peptide sequence in all intracellular oomycete effectors characterized to date (reviewed in Birch *et al.*, 2006; Ellis *et al.*, 2006). This motif is reminiscent of a sequence pattern present at a comparable position in secreted virulence factors of the intracellular mammalian pathogen, *Plasmodium falciparum*, the causal agent of malaria. In *P. falciparum*, this peptide motif is essential for proteins to cross the host membrane during export from the parasite vacuole into the human erythrocyte (Hiller *et al.*, 2004; Marti *et al.*, 2004).

2. Database mining: a trendy approach for the identification of candidate effectors

The availability of resources like comprehensive microbial cDNA expressed sequence tag (EST) collections (Randall *et al.*, 2005; Soanes & Talbot, 2006) or, as in the case of *M. grisea* and *Phytophthora infestans*, full or partial genome sequences (Dean *et al.*, 2005; Randall *et al.*, 2005), provides an alternative approach to searching for candidate secreted effector polypeptides. Biocomputational analysis allows the predicition of secreted proteins based on the presence of canonical N-terminal secretion signals (Torto *et al.*, 2003; Catanzariti *et al.*, 2006). Additionally, putative effectors can be identified genetically based on the role of the gene products as avirulence factors that are recognized by matching resistance (*R*) genes in the host. In this case, the ability of the proteins to trigger host cell death can be exploited for genetic mapping and subsequent gene cloning. Examples of this approach include the recent identification of *AvrL567* from the flax rust fungus, *M. lini* (Dodds *et al.*, 2004), *ATR1* and *ATR13* from the biotrophic oomycete *Hyaloperonospora parasitica* (Allen *et al.*, 2004; Rehmany *et al.*, 2005) as well as *Avr3a* and *Avr1b-1* encoded by the hemibiotrophic oomycetes *P. infestans* and *P. sojae*, respectively (Shan *et al.*, 2004; Armstrong *et al.*, 2005). In a recent study, computational prediction of haustorially expressed secreted proteins was combined with subsequent genetic mapping in relation to known *Avr* loci to identify several novel effectors of the flax rust fungus (Catanzariti *et al.*, 2006). All these genes encode relatively small proteins with no significant homology to existing database entries. Except for AvrP123, which contains a motif characteristic of the Kazal family of serine protease inhibitors (Catanzariti *et al.*, 2006), the primary amino acid sequence of these polypeptides does not provide any clue to their biochemical function. As mentioned in the previous section for the bacterial effector AvrPtoB (Janjusevic *et al.*, 2006), structural analysis might help to unravel the potential role of these polypeptides.

Based on comparison of EST and genomic sequences from different pathogens, it appears that most of these effectors are restricted to a narrow phylogenetic spectrum, being either species- or genus-specific (Allen *et al.*, 2004; Dodds *et al.*, 2004; Shan *et al.*, 2004; Armstrong *et al.*, 2005; Kemen *et al.*, 2005; Rehmany *et al.*, 2005; Catanzariti *et al.*, 2006). One characteristic of the genes encoding effectors that function as avirulence determinants is that they appear to evolve rapidly, that is the respective genes are under diversifying selection (Table 2). This may reflect the ongoing molecular 'arms race' between host and pathogen – a process of coevolution in which the pathogen evolves to evade recognition by the host via selection-driven alterations of its effector molecules while the host subsequently evolves novel recognition specificities that allow it to keep track of the pathogen's changing molecular camouflage (Allen *et al.*, 2004; Maor & Shirasu, 2005). The rapid coevolution of matching *Avr–R* gene pairs in the context of existing compatible plant–microbe interactions may in part explain the restriction of pathogen effectors to a narrow phylogenetic spectrum (Rep, 2005).

3. Special effect(or)s in the plant–microbe show

In two closely related rust fungi, *U. fabae* and *U. striatus*, recent immunolocalization studies suggest that a haustorially expressed and secreted glycoprotein, RTP1p, eventually localizes in the nuclei of infected mesophyll cells of their respective host plants, *V. faba* and *M. truncatula* (Kemen *et al.*, 2005; Fig. 5). Similarly, one protein of *P. infestans* carrying the oomycete-specific RXLR motif mentioned in Section IV.1 bears a functional nuclear localization signal (Kamoun, 2006). These findings support previous speculation that effector polypeptides of plant parasites might, in part, affect nuclear functions (e.g. transcription) to reprogram host cells for biotrophy.

There is evidence that, at least in one instance, effector proteins may lack a typical N-terminal signal peptide. Two members of the *B. graminis Avr* gene family (Avr_{k1} and Avr_{a10}) that were isolated by map-based cloning encode sequence-related short polypeptides lacking prototypical signal peptides but bearing distinct molecular features that may enable them to cross lipid bilayers (Ridout, 2004). A likely localization of these effectors in the host cytoplasm was inferred from the fact that members of the MLA family of barley resistance proteins

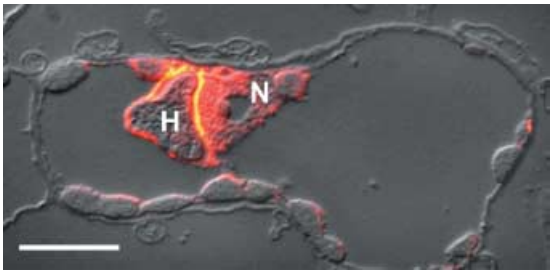

Fig. 5 Immunofluorescence labelling (orange-red), illustrating translocation of the glycoprotein *Uf*-RTP1p from a haustorium (H) of the bean rust *Uromyces fabae* into the cytoplasm and nucleus (N) of an infected *Vicia faba* cell. Bar, 10 μm. (Image provided by Eric Kemen *et al.* University of Konstanz, Konstanz, Germany.)

are cytosolic polypeptides (Bieri *et al.*, 2004), suggesting that recognition of matching Avr proteins such as AVR_{a10} takes place in this subcellular compartment. This example illustrates that the computational procedures commonly used to identify effectors based on the presence of archetypal signal peptides might be insufficient to capture the full diversity of effectors encoded by fungal/oomycete plant parasites. It remains to be shown whether Avr_{k1} and Avr_{a10} represent unusual exceptions or whether effectors bearing canonical secretion signals represent just the tip of the effector iceberg.

4. Biotroph genomes: opening a Pandora's box of effectors

How many secreted polypeptides might be encoded by (hemi-)biotrophic fungal/oomycete pathogens? Comprehensive computational analyses revealed that the genomes of various *Phytophthora* species each encode more than 100 potentially secreted proteins carrying the RXLR motif, suggesting that a considerable number of *Phytophthora* effectors may act inside host cells (Kamoun, 2006). Likewise, biocomputational analysis suggests that the genome of *M. grisea* encodes more than 700 secreted proteins (Dean *et al.*, 2005). Given that at least some effectors are not included in this calculation, because of the absence of recognizable secretion motifs (described earlier) or misidentification of putative reading frames, the true number might be even higher. In conclusion, it appears that the effector repertoire of these fungal and oomycete pathogens is considerably more complex than that of phytopathogenic bacteria. This likely reflects their more sophisticated infection process, including the necessity for host cell entry and subsequent accommodation of their highly differentiated infection structures by living host cells. Recently, a novel database, PHI-base, was established that stores verified sequences of fungal and oomycete effector proteins (Winnenburg *et al.*, 2006; http://www.phi-base.org).

It should be noted that the effector arsenal of microbial pathogens is not restricted to polypeptides but also includes secondary metabolites. A well-known example is the compound coronatine, a metabolite synthesized by plant–pathogenic *Pseudomonas* bacteria that mimics the plant defence signalling molecule jasmonic acid. Given the antagonistic relationship of salicylic acid and jasmonic acid-dependent defence pathways in Arabidopsis, it is currently thought that the pathogen-derived coronatine serves a role in repressing salicylic acid-mediated responses (reviewed in Nomura *et al.*, 2005). The rice blast fungus, *M. grisea*, encodes ACE1, a putative hybrid protein between a polyketide synthase and a nonribosomal polypeptide synthetase that is assumed to catalyse the synthesis of a secreted secondary metabolite that may likewise contribute to fungal pathogenesis (Bohnert *et al.*, 2004). Intriguingly, a cytochalasin-like molecule secreted by *M. grisea* during spore germination and invasive growth within host tissues appears to suppress the basal resistance of *Digitaria* plants to nonadapted isolates of this pathogen, although whether this involves perturbation of the host actin cytoskeleton remains unclear (Tsurushima *et al.*, 2005).

V. Plant factors for compatibility

1. Plant compatibility genes: meeting the needs of your parasite

It is likely that the complex molecular interplay between an invading (hemi-)biotrophic pathogen and its respective host plant involves highly specific molecular, such as protein–protein, interactions. These might, for example, take place on the leaf surface before cell wall penetration, at the stage of infection structure accommodation or during manipulation of the plant cell via secreted effector molecules. Consequently, the lack of appropriate host target molecules may result in failure of the pathogen to control the plant effectively, and ultimately the premature termination of pathogenesis. Thus, lack of essential host factors could, in principle, lead to resistance against a given pathogen species without the constitutive activation of plant defence responses. Unless the host factor was the target of multiple pathogen species, this type of disease resistance is therefore assumed to be specific for a pathogen species/class and is most likely not isolate-specific. Since resistance would be brought about by the loss of a single gene function, it is expected to be recessively inherited. Resistance of plants to potyviruses through lack of the eukaryotic translation elongation factor isoform, eIF(iso)E4, serves as a paradigm for this type of immunity (reviewed in Robaglia & Caranta, 2006). Apart from possible deficiency of an effector target, loss of a host factor may cause an altered physiological state that indirectly hampers microbial pathogenesis.

A range of examples of monogenic, recessively inherited and pathogen-specific disease resistance loci have been reported from both monocot and dicot plant species (Table 3). Probably one of the best-studied examples is powdery mildew resistance mediated by loss-of-function alleles of the barley *mildew resistance locus o* (*Mlo*). Presence of wild-type *Mlo* is required for the successful entry of powdery mildew sporelings into epidermal cells of their respective host, barley. Barley *Mlo* encodes a member of a novel plant-specific family of integral membrane proteins (Büschges *et al.*, 1997; Devoto *et al.*, 1999, 2003). Although the exact biochemical function of MLO in the context of host cell entry is as yet unknown, one possibility is that it controls exocytotic processes and that the powdery mildew pathogen corrupts MLO function for defence suppression (Panstruga, 2005).

2. The usual suspect: Arabidopsis as a model to dissect host contributions to compatibility

Like barley *Mlo*, Arabidopsis *powdery mildew resistant* (*pmr*) mutants are recessively inherited loss-of-function mutants

Table 3 Potential host 'compatibility factors' of (hemi-)biotrophic pathogens[a]

Gene	Plant species	*cichoraceorum* Pathogen	Disease	Biochemical function of encoded protein	Reference
CHR(1–3?)	*Arabidopsis thaliana*	*Colletotrichum higginsianum*	Anthracnose	?	D. Birker and R. O'Connell (unpublished)
DMR1–DMR6	*Arabidopsis thaliana*	*Hyaloperonospora parasitica*	Downy mildew	?	Van Damme *et al.* (2005)
ER1	*Pisum sativum*	*Erysiphe pisi*	Powdery mildew	?	Timmerman *et al.* (1994)
OL2	*Lycopersicon esculentum*	*Oidium lycopersici*	Powdery mildew	?	Ciccarese *et al.* (1998)
Mlo	*Hordeum vulgare*	*Blumeria graminis*	Powdery mildew	Putative modulator of exocytosis	Büschges *et al.* (1997); Panstruga (2005)
PMR1, PMR2	*Arabidopsis thaliana*	*Golovinomyces* (formerly *Erysiphe*) *cichoracearum*	Powdery mildew	?	Vogel & Somerville (2000)
PMR2	*Arabidopsis thaliana*	*Golovinomyces* (formerly *Erysiphe*) *cichoracearum*	Powdery mildew	MLO ortholog (see Section V.1)	Consonni *et al.* (2006)
PMR4	*Arabidopsis thaliana*	*Golovinomyces* (formerly *Erysiphe*) *cichoracearum*	Powdery mildew	Callose synthase	Jacobs *et al.* (2003); Nishimura *et al.* (2003)
PMR5	*Arabidopsis thaliana*	*Golovinomyces* (formerly *Erysiphe*) *cichoracearum*	Powdery mildew	Protein of unknown function	Vogel *et al.* (2004)
PMR6	*Arabidopsis thaliana*	*Golovinomyces* (formerly *Erysiphe*) *cichoracearum*	Powdery mildew	Pectate lyase-like protein	Vogel *et al.* (2002)

[a]Based on genuine plant mutants/natural plant variants, excluding results based solely on transient gene expression (Hückelhoven, 2005).

that provide enhanced disease resistance to *G. cichoracearum*, a powdery mildew of cruciferous plants that also colonizes *A. thaliana*. Six *pmr* loci (*pmr1–6*) have been identified in a forward genetic screen for loss of *G. cichoracearum* sporulation (Vogel & Somerville, 2000; Table 3) and four of the respective genes have been isolated by map-based cloning. *PMR2* encodes an ortholog of the barley *Mlo* gene referred to in the previous section, *AtMLO2*, suggesting that *mlo*-based resistance is not restricted to the monocot barley and is a general feature of higher plant species (Consonni *et al.*, 2006). The gene affected in the *pmr4* mutant, *PMR4*, encodes a callose synthase isoform (GSL5) that is essential for callose deposition at wound and biotic stress sites (Jacobs *et al.*, 2003; Nishimura *et al.*, 2003). The finding that absence of callose results in enhanced disease resistance to the powdery mildew pathogen was a surprising result, as callose has been assumed for a long time to play a major role as a physical barrier to fungal entry, for example by its presence in local cell wall reinforcements (papillae). Although the molecular basis for *pmr4*-conditioned resistance remains unresolved, it is intriguing that the nine *pmr4* alleles recovered from genetic screens are predicted null mutants characterized by either premature stop codons or a frame shift in the coding region (Nishimura *et al.*, 2003). This suggests that neither PMR4 catalytic activity nor its immediate biochemical reaction product, callose, plays a significant role in mediating disease susceptibility but rather presence of the PMR4 protein *per se*. The remaining two loci, *PMR5* and *PMR6*, encode a protein of unknown function and a pectate lyase, respectively (Vogel *et al.*, 2002; 2004; also discussed earlier). In both cases, alterations in the cell wall composition of the respective mutants have been reported. Despite this finding, a convincing model to explain resistance is lacking to date. Taken together, the collection of *pmr* mutants has not yet revealed a conclusive concept that may help to understand host cell manipulation during powdery mildew pathogenesis.

Genetic screens in *A. thaliana* have not only revealed genes required for compatibility with powdery mildew pathogens but also loci that are essential for susceptibility to the causal agent of the downy mildew disease, *H. parasitica*. In total, six downy mildew resistance loci (*dmr1–6*) have been identified, of which three confer resistance without constitutive defence responses (Van Damme *et al.*, 2005; Table 3). Similarly, an ongoing genetic screen for Arabidopsis mutants showing loss of susceptibility to the hemibiotrophic pathogen *C. higginsianum* has so far revealed three candidate *chr* (*C. higginsianum*-resistant) mutants among 100 000 tested M2 plants (D. Birker & R. O'Connell, unpublished). In addition to these candidates, the *dmr6*, *pmr2* and *pmr6* mutants also exhibit either strong (*dmr6*) or partial (*pmr2*, *pmr6*) resistance to the anthracnose fungus, suggesting that some plant genes required to support infection by obligate biotrophs also play a role in susceptibility to this hemibiotroph.

VI. Future directions and opportunities

The comprehensive analysis of compatibility between plants and haustorium-forming biotrophs continues to be severely hampered by the lack of reliable, stable transformation systems for these organisms. Thus, experimental work with obligate biotrophs is usually either descriptive or indirect (e.g. based on heterologous gene expression). Hemibiotrophic pathogens that can be both cultured *in vitro* and genetically manipulated therefore represent an attractive experimental alternative to study the principles of plant–microbe compatibility. For example, *C. higginsianum* in combination with *A. thaliana* emerges as an excellent model pathosystem in which both partners are genetically tractable (Narusaka *et al.*, 2004; O'Connell *et al.*, 2004). However, compared with the well-studied host plant, the molecular and genetic tool box on the pathogen side is currently limited and a full genome sequence is urgently required for this organism. Likewise, work with obligate biotrophs would benefit greatly from the availability of full genome sequences and comprehensive EST datasets that include the intracellular biotrophic phase (Zhang *et al.*, 2005). In both cases, this would allow the biocomputational prediction of secreted effector proteins, as previously demonstrated for *P. infestans* (Torto *et al.*, 2003).

Yeast-based genetic screens to identify pathogen-derived cDNAs encoding proteins with functional N-terminal secretion signals offer an interesting alternative to the commonly used bioinformatic approaches (Jacobs *et al.*, 1997; Chen & Leder, 1999). These functional selection procedures may capture secretion signals that escape current prediction algorithms. Additionally, yeast-based assays could be exploited to systematically search for pathogen effectors that are able to suppress programmed cell death (Xu & Reed, 1998). Based on the data obtained with bacterial cell death-suppressing effector proteins (Abramovitch *et al.*, 2003; Jamir *et al.*, 2004), such an approach appears feasible. Cell death suppression might be even more important for (hemi-)biotrophic pathogens that breach plant cell walls and establish feeding structures inside host cells. Functional analysis of candidate cDNAs derived from obligate biotrophs is currently largely restricted to heterologous *in planta* expression studies (Dodds *et al.*, 2004; Allen *et al.*, 2005; Rehmany *et al.*, 2005; Catanzariti *et al.*, 2006). However, genetically accessible hemibiotrophs such as *Colletotrichum* spp. or *Phythophthora* spp. might be exploited as surrogate vehicles to deliver microbial effectors in a more native manner.

Laser capture microdissection is a relatively novel tool that allows sampling of biological material at the cellular or even subcellular level (Kehr, 2003). This technique may enable scientists to recover, at medium to high throughput, macromolecules (e.g. RNA and/or protein) from individual infected plant cells or even microbial infection structures such as infection hyphae or haustoria. The latter are generally difficult to retrieve as intact entities by conventional separation procedures and therefore, with a few exceptions (Hahn & Mendgen, 1997; Catanzariti *et al.*, 2006), have not yet been subject to in-depth molecular analysis. RNA and protein samples obtained by microdissection of infection hyphae or haustoria may be used to generate cDNA libraries, as probes for cDNA microarrays, or to perform proteomic studies. This technique may thus represent a powerful means to obtain information about gene expression patterns and/or protein complements of fungal and oomycete infection structures.

While the last decade has seen remarkable progress in our understanding of biotrophic nutrition (Voegele & Mendgen, 2003), in the next 5 years we can expect exciting new insights into other fundamental aspects of intracellular biotrophy, for example the mechanisms by which these pathogens suppress host cell death and defence responses and induce host cells to accommodate their infection structures and elaborate a specialized interface with them.

Acknowledgements

We are grateful to Volker Lipka, Eric Kemen, Ralf Voegele and Kurt Mendgen for critical reading of the manuscript. We also thank George Barron, Raffaella Carzaniga, Eric Kemen, Serry Koh, Kurt Mendgen and Martina Stark-Urnau for providing micrographs. Work in the laboratories of the authors is supported by grants from the Max-Planck society (RP, RO'C) and the Deutsche Forschungsgemeinschaft (DFG, RP).

References

Abramovitch RB, Kim YJ, Chen SR, Dickman MB, Martin GB. 2003. *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO Journal* **22**: 60–69.

Agrawal GK, Rakwal R, Tamogami S, Yonekura M, Kubo A, Saji H. 2002. Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. *Plant Physiology and Biochemistry* **40**: 1061–1069.

Alfano JR, Collmer A. 2004. Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. *Annual Review of Phytopathology* **42**: 385–414.

Allen RL, Bittner-Eddy PD, Grenville-Briggs LJ, Meitz JC, Rehmany AP, Rose LE, Beynon JL. 2004. Host-parasite coevolutionary conflict between *Arabidopsis* and downy mildew. *Science* **306**: 1957–1960.

Armstrong MR, Whisson SC, Pritchard L, Bos JIB, Venter E, Avrova AO, Rehmany AP, Bohme U, Brooks K, Cherevach I, Hamlin N, White B, Frasers A, Lord A, Quail MA, Churcher C, Hall N, Berriman M, Huang S, Kamoun S, Beynon JL, Birch PRJ. 2005. An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *Proceedings of the National Academy of Sciences, USA* **102**: 7766–7771.

Assaad FF, Qiu JL, Youngs H, Ehrhardt D, Zimmerli L, Kalde M, Wanner G, Peck SC, Edwards H, Ramonell K, Somerville CR, Thordal-Christensen H. 2004. The PEN1 syntaxin defines a novel cellular compartment upon fungal attack and is required for the timely assembly of papillae. *Molecular Biology of the Cell* **15**: 5118–5129.

Baka ZA, Larous L, Losel DM. 1995. Distribution of ATPase activity at the host–pathogen interfaces of rust infections. *Physiological and Molecular Plant Pathology* **47**: 67–82.

Balestrini R, Bonfante P. 2005. The interface compartment in arbuscular mycorrhizae: a special type of plant cell wall? *Plant Biosystems* **139**: 8–15.

Bauer R, Mendgen K, Oberwinkler F. 1995. Cellular interaction of the smut fungus *Ustacystis waldsteiniae*. *Canadian Journal of Botany* **73**: 867–883.

Bechinger C, Giebel KF, Schnell M, Leiderer P, Deising HB, Bastmeyer M. 1999. Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. *Science* **285**: 1896–1899.

Bhat RA, Miklis M, Schmelzer E, Schulze-Lefert P, Panstruga R. 2005. Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane microdomain. *Proceedings of the National Academy of Sciences, USA* **102**: 3135–3140.

Bhat RA, Panstruga R. 2005. Lipid rafts in plants. *Planta* **223**: 5–19.

Bieri S, Mauch S, Shen QH, Peart J, Devoto A, Casais C, Ceron F, Schulze S, Steinbiss HH, Shirasu K, Schulze-Lefert P. 2004. RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. *Plant Cell* **16**: 3480–3495.

Birch PRJ, Rehmany AP, Pritchard L, Kamoun S, Beynon JL. 2006. Trafficking arms: oomycete effectors enter host plant cells. *Trends in Microbiology* **14**: 8–11.

Bishop JG, Ripoll DR, Bashir S, Damasceno CMB, Seeds JD, Rose JKC. 2005. Selection on glycine beta-1,3-endoglucanase genes differentially inhibited by a *Phytophthora* glucanase inhibitor protein. *Genetics* **169**: 1009–1019.

Bohnert HU, Fudal I, Dioh W, Tharreau D, Notteghem JL, Lebrun MH. 2004. A putative polyketide synthase peptide synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. *Plant Cell* **16**: 2499–2513.

Bonifacino JS, Glick BS. 2004. The mechanisms of vesicle budding and fusion. *Cell* **116**: 153–166.

Both M, Csukai M, Stumpf MPH, Spanu PD. 2005a. Gene expression profiles of *Blumeria graminis* indicate dynamic changes to primary metabolism during development of an obligate biotrophic pathogen. *Plant Cell* **17**: 2107–2122.

Both M, Eckert SE, Csukai M, Muller E, Dimopoulos G, Spanu PD. 2005b. Transcript profiles of *Blumeria graminis* development during infection reveal a cluster of genes that are potential virulence determinants. *Molecular Plant–Microbe Interactions* **18**: 125–133.

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Töpsch S, Vos P, Salamini F, Schulze-Lefert P. 1997. The barley *Mlo* gene: a novel control element of plant pathogen resistance. *Cell* **88**: 695–705.

Catanzariti AM, Dodds PN, Lawrence GJ, Ayliffe MA, Ellis JG. 2006. Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. *Plant Cell* **18**: 243–256.

Celio GJ, Mims CW, Richardson EA. 2004. Ultrastructure and immunocytochemistry of the host–pathogen interface in *Poinsettia* leaves infected with powdery mildew. *Canadian Journal of Botany* **82**: 421–429.

Chang JH, Goel AK, Grant SR, Dangl JL. 2004. Wake of the flood: ascribing functions to the wave of type III effector proteins of phytopathogenic bacteria. *Current Opinion in Microbiology* **7**: 11–18.

Chen HC, Leder P. 1999. A new signal sequence trap using alkaline phosphatase as a reporter. *Nucleic Acids Research* **27**: 1219–1222.

Ciccarese F, Amenduni M, Schiavone D, Cirulli M. 1998. Occurrence and inheritance of resistance to powdery mildew (*Oidium lycopersici*) in *Lycopersicon* species. *Plant Pathology* **47**: 417–419.

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Hückelhoven R, Stein M, Freialdenhoven A, Somerville SC, Schulze-Lefert P. 2003. SNARE-protein-mediated disease resistance at the plant cell wall. *Nature* **425**: 973–977.

Consonni C, Humphry ME, Hartmann HA, Livaja M, Durner J, Westphal L, Vogel J, Lipka V, Kemmerling B, Schulze-Lefert P, Somerville SC, Panstruga R . 2006. Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetic* **38**: 716–720.

De Lorenzo G, D'Ovidio R, Cervone F. 2001. The role of polygalacturonase-inhibiting proteins (PGIPS) in defense against pathogenic fungi. *Annual Review of Phytopathology* **39**: 313–335.

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan HQ, Read ND, Lee YH, Carbone I, Brown D, Oh YY, Donofrio N, Jeong JS, Soanes DM, Djonovic S, Kolomiets E, Rehmeyer C, Li WX, Harding M, Kim S, Lebrun MH, Bohnert H, Coughlan S, Butler J, Calvo S, Ma LJ, Nicol R, Purcell S, Nusbaum C, Galagan JE, Birren BW . 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* **434**: 980–986.

Deising H, Siegrist J. 1995. Chitin deacetylase activity of the rust *Uromyces viciae fabae* is controlled by fungal morphogenesis. *FEMS Microbiology Letters* **127**: 207–211.

Deising HB, Werner S, Wernitz M. 2000. The role of fungal appressoria in plant infection. *Microbes and Infection* **2**: 1631–1641.

Devoto A, Hartmann HA, Piffanelli P, Elliott C, Simmons C, Taramino G, Goh CS, Cohen FE, Emerson BC, Schulze-Lefert P, Panstruga R. 2003. Molecular phylogeny and evolution of the plant-specific seven-transmembrane MLO family. *Journal of Molecular Evolution* **56**: 77–88.

Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, Schulze-Lefert P. 1999. Topology, subcellular localization, and sequence diversity of the *Mlo* family in plants. *Journal of Biological Chemistry* **274**: 34993–35004.

Dodds PN, Lawrence GJ, Catanzariti AM, Ayliffe MA, Ellis JG. 2004. The *Melampsora lini AvrL567* avirulence genes are expressed in haustoria and their products are recognized inside plant cells. *Plant Cell* **16**: 755–768.

Douchkov D, Nowara D, Zierold U, Schweizer P. 2005. A high-throughput gene-silencing system for the functional assessment of defense-related genes in barley epidermal cells. *Molecular Plant–Microbe Interactions* **18**: 755–761.

El Gueddari NE, Rauchhaus U, Moerschbacher BM, Deising HB. 2002. Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. *New Phytologist* **156**: 103–112.

Ellis J, Catanzariti AM, Dodds P. 2006. The problem of how fungal and oomycete avirulence proteins enter plant cells. *Trends in Plant Science* **11**: 61–63.

Enkerli K, Hahn MG, Mims CW. 1997. Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. *Canadian Journal of Botany* **75**: 1493–1508.

Eskandari S, Wright EM, Kreman M, Starace DM, Zampighi GA. 1998. Structural analysis of cloned plasma membrane proteins by freeze-fracture electron microscopy. *Proceedings of the National Academy of Sciences, USA* **95**: 11235–11240.

Fasters MK, Daniels U, Moerschbacher BM. 1993. A simple and reliable method for growing the wheat stem rust fungus, *Puccinia graminis* f.sp. *tritici*, in liquid culture. *Physiological and Molecular Plant Pathology* **42**: 259–265.

Feys BJ, Wiermer M, Bhat RA, Moisan LJ, Medina-Escobar N, Neu C, Cabral A, Parker JE. 2005. Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell* **17**: 2601–2613.

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**: 3489–3499.

Gil F, Gay JL. 1977. Ultrastructural and physiological properties of host interfacial components of haustoria of *Erysiphe pisi in vivo* and *in vitro*. *Physiological Plant Pathology* **10**: 1–12.

Gniwotta F, Vogg G, Gartmann V, Carver TLW, Riederer M, Jetter R. 2005. What do microbes encounter at the plant surface? Chemical composition of pea leaf cuticular waxes. *Plant Physiology* **139**: 519–530.

Gold RE, Mendgen K. 1984. Cytology of basidiospore germination, penetration, and early colonization of *Phaseolus vulgaris* by *Uromyces appendiculatus* var. *appendiculatus*. *Canadian Journal of Botany* **62**: 1989–2002.

Green JR, Carver TLW, Gurr SJ. 2002. The formation and function of infection and feeding structures. In: Belanger RR, Bushnell WR, Dik AJ, Carver TLW, eds. *The powdery mildews, a comprehensive treatise*. St Paul, MN, USA: APS Press, 66–82.

Green JR, Pain NA, Cannell ME, Jones GL, Leckie CP, McCready S, Mendgen K, Mitchell AJ, Callow JA, O'Connell RJ. 1995. Analysis of differentiation and development of the specialized infection structures formed by biotrophic fungal plant pathogens using monoclonal antibodies. *Canadian Journal of Botany* **73**: S408–S417.

Hahn M, Mendgen K. 1992. Isolation by ConA binding of haustoria from different rust fungi and comparison of their surface qualities. *Protoplasma* **170**: 95–103.

Hahn M, Mendgen K. 1997. Characterization of *planta* induced rust genes isolated from a haustorium-specific cDNA library. *Molecular Plant–Microbe Interactions* **10**: 427–437.

Hahn M, Neef U, Struck C, Gottfert M, Mendgen K. 1997. A putative amino acid transporter is specifically expressed in haustoria of the rust fungus *Uromyces fabae*. *Molecular Plant–Microbe Interactions* **10**: 438–445.

Hajlaoui MR, Benhamou N, Belanger RR. 1991. Cytochemical aspects of fungal penetration, haustorium formation and interfacial material in rose leaves infected by *Sphaerotheca pannosa* var. *rosae*. *Physiological and Molecular Plant Pathology* **38**: 341–355.

Ham KS, Wu SC, Darvill AG, Albersheim P. 1997. Fungal pathogens secrete an inhibitor protein that distinguishes isoforms of plant pathogenesis-related endo-beta-1,3-glucanases. *Plant Journal* **11**: 169–179.

Harder DE, Chong J. 1984. Structure and physiology of haustoria. In: Bushnell WR, Roelfs P, eds. *The Cereal Rusts*, Vol. I. *Origins, specificity, structure, and physiology*. Orlando, FL, USA: Academic Press, 431–476.

Harder DE, Mendgen K. 1982. Filipin-sterol complexes in bean rust-fungal and oat crown rust–fungal plant interactions – freeze-etch electron microscopy. *Protoplasma* **112**: 46–54.

Harrison MJ, Dewbre GR, Liu JY. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisiton of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* **14**: 2413–2429.

Hauck P, Thilmony R, He SY. 2003. A *Pseudomonas syringae* type III effector suppresses cell wall-based extracellular defense in susceptible Arabidopsis plants. *Proceedings of the National Academy of Sciences, USA* **100**: 8577–8582.

Heath MC, Howard RJ, Valent B, Chumley FG. 1992. Ultrastructural interactions of one strain of *Magnaporthe grisea* with goosegrass and weeping lovegrass. *Canadian Journal of Botany* **70**: 779–787.

Heath MC, Skalamera D. 1997. Cellular interactions between plants and biotrophic fungal parasites. *Advances in Botanical Research* **24**: 195–225.

Heese A, Ludwig AA, Jones JDG. 2005. Rapid phosphorylation of a syntaxin during the Avr9/Cf-9-race-specific signaling pathway. *Plant Physiology* **138**: 2406–2416.

Hepler PK, Vidali L, Cheung AY. 2001. Polarized cell growth in higher plants. *Annual Review of Cell and Developmental Biology* **17**: 159–187.

Herbert C, O'Connell R, Gaulin E, Salesses V, Esquerré-Tugayé M-T, Dumas B. 2004. Production of a cell wall-associated endopolygalacturonase by *Colletotrichum lindemuthianum* and pectin degradation during bean infection. *Fungal Genetics and Biology* **41**: 140–147.

Hiller NL, Bhattacharjee S, van Ooij C, Liolios K, Harrison T, Lopez-Estrano C, Haldar K. 2004. A host-targeting signal in virulence proteins reveals a secretome in malarial infection. *Science* **306**: 1934–1937.

Hippe-Sanwald S, Marticke KH, Kieliszewski MJ, Somerville SC. 1994. Immunogold localization of THRGP-like epitopes in the haustorial interface of obligate, biotrophic fungi on monocots. *Protoplasma* **178**: 138–155.

Howard RJ, Ferrari MA, Roach DH, Money NP. 1991. Penetration of hard substrates by a fungus employing enormous turgor pressures. *Proceedings of the National Academy of Sciences, USA* **88**: 11281–11284.

Hu GG, Rijkenberg FHJ. 1998. Subcellular localization of beta-1,3-glucanase in *Puccinia recondita* f.sp. *tritici*-infected wheat leaves. *Planta* **204**: 324–334.

Hückelhoven R. 2005. Powdery mildew susceptibility and biotrophic infection strategies. *FEMS Microbiology Letters* **245**: 9–17.

Hückelhoven R, Kogel KH. 2003. Reactive oxygen intermediates in plant–microbe interactions: Who is who in powdery mildew resistance? *Planta* **216**: 891–902.

Jacobs KA, Collins-Racie LA, Colbert M, Duckett M, Golden-Fleet M, Kelleher K, Kriz R, LaVallie ER, Merberg D, Spaulding V, Stover J, Williamson MJ, McCoy JM. 1997. A genetic selection for isolating cDNAs encoding secreted proteins. *Gene* **198**: 289–296.

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, Fincher GB. 2003. An Arabidopsis callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell* **15**: 2503–2513.

Jakupovic M, Heintz M, Reichmann P, Mendgen K, Hahn M. 2006. Microarray analysis of expressed sequence tags from haustoria of the rust fungus *Uromyces fabae*. *Fungal Genetics and Biology* **43**: 8–19.

Jamir Y, Guo M, Oh HS, Petnicki-Ocwieja T, Chen SR, Tang XY, Dickman MB, Collmer A, Alfano JR . 2004. Identification of *Pseudomonas syringae* type III effectors that can suppress programmed cell death in plants and yeast. *Plant Journal* **37**: 554–565.

Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE. 2006. A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. *Science* **311**: 222–226.

Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B. 2000. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO Journal* **19**: 4004–4014.

Jin QL, Thilmony R, Zwiesler-Vollick J, He SY. 2003. Type III protein secretion in *Pseudomonas syringae*. *Microbes and Infection* **5**: 301–310.

Kahmann R, Kämper J. 2004. *Ustilago maydis*: how its biology relates to pathogenic development. *New Phytologist* **164**: 31–42.

Kamoun S. 2006. A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology* **44**: 2.1–2.2.20.

Kehr J. 2003. Single cell technology. *Current Opinion in Plant Biology* **6**: 617–621.

Kemen E, Kemen AC, Rafiqi M, Hempel U, Mendgen K, Hahn M, Voegele RT. 2005. Identification of a protein from rust fungi transferred from haustoria into infected plant cells. *Molecular Plant–Microbe Interactions* **18**: 1130–1139.

Knauf GM, Welter K, Muller M, Mendgen K. 1989. The haustorial host–parasite interface in rust-infected bean leaves after high-pressure freezing. *Physiological and Molecular Plant Pathology* **34**: 519–530.

Koh S, André A, Edwards H, Ehrhardt D, Somerville S. 2005. *Arabidopsis thaliana* subcellular responses to compatible *Erysiphe cichoracearum* infections. *Plant Journal* **44**: 516–529.

Latunde-Dada AO, O'Connell RJ, Nash C, Pring RJ, Lucas JA, Bailey JA. 1996. Infection process and identity of the hemibiotrophic anthracnose fungus (*Colletotrichum destructivum*) from cowpea (*Vigna unguiculata*). *Mycological Research* **100**: 1133–1141.

Leckie CP, Callow JA, Green JR. 1995. Reorganization of the endoplasmic-reticulum in pea leaf epidermal cells infected by the powdery mildew fungus *Erysiphe pisi*. *New Phytologist* **131**: 211–221.

Lefebvre B, Batoko H, Duby G, Boutry M. 2004. Targeting of a *Nicotiana plumbaginifolia* H^+-ATPase to the plasma membrane is not by default and requires cytosolic structural determinants. *Plant Cell* **16**: 1772–1789.

Lewis DH. 1973. Concepts in fungal nutrition and origin of biotrophy. *Biology Reviews of the Cambridge Philosophical Society* **48**: 261–278.

Link T, Lohaus G, Heiser I, Mendgen K, Hahn M, Voegele RT. 2005. Characterization of a novel NADP(+)-dependent D-arabitol dehydrogenase from the plant pathogen *Uromyces fabae*. *Biochemical Journal* **389**: 289–295.

Lipka V, Dittgen J, Bednarek P, Bhat R, Wiermer M, Stein M, Landtag J, Brandt W, Rosahl S, Scheel D, Llorente F, Molina A, Parker J, Somerville S, Schulze-Lefert P. 2005. Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science* **310**: 1180–1183.

Lipka V, Panstruga R. 2005. Dynamic cellular responses in plant–microbe interactions. *Current Opinion in Plant Biology* **8**: 625–631.

Liu JY, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ. 2003. Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *Plant Cell* **15**: 2106–2123.

Liu JY, Blaylock LA, Harrison MJ. 2004. cDNA arrays as a tool to identify mycorrhiza-regulated genes: identification of mycorrhiza-induced genes that encode or generate signaling molecules implicated in the control of root growth. *Canadian Journal of Botany* **82**: 1177–1185.

Mackie AJ, Roberts AM, Callow JA, Green JR. 1991. Molecular differentiation in pea powdery mildew haustoria – identification of a 62-kDa N-linked glycoprotein unique to the haustorial plasma membrane. *Planta* **183**: 399–408.

Mackie AJ, Roberts AM, Green JR, Callow JA. 1993. Glycoproteins recognized by monoclonal antibodies UB7, UB8 and UB10 are expressed early in the development of pea powdery mildew haustoria. *Physiological and Molecular Plant Pathology* **43**: 135–146.

Maldonado-Mendoza IE, Dewbre GR, Blaylock L, Harrison MJ. 2005. Expression of a xyloglucan endotransglucosylase/hydrolase gene, *Mt-XTH1*, from *Medicago truncatula* is induced systemically in mycorrhizal roots. *Gene* **345**: 191–197.

Manners JM, Gay JL. 1983. The host–parasite interface and nutrient transfer in biotrophic parasitism. In: Callow JA, ed. *Biochemical plant pathology.* Chichester, UK: John Wiley, 163–195.

Maor R, Shirasu K. 2005. The arms race continues: battle strategies between plants and fungal pathogens. *Current Opinion in Microbiology* **8**: 399–404.

Marti M, Good RT, Rug M, Knuepfer E, Cowman AF. 2004. Targeting malaria virulence and remodeling proteins to the host erythrocyte. *Science* **306**: 1930–1933.

Marticke KH, Reisener HJ, Fischer R, Hippe-Sanwald S. 1998. *In situ* detection of a fungal glycoprotein-elicitor in stem rust-infected susceptible and resistant wheat using immunogold electron microscopy. *European Journal of Cell Biology* **76**: 265–273.

Mauch F, Staehelin LA. 1989. Functional implications of the subcellular-localization of ethylene-induced chitinase and beta-1,3-glucanase in bean-leaves. *Plant Cell* **1**: 447–457.

Mendgen K, Hahn M, Deising H. 1996. Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annual Review of Phytopathology* **34**: 367–386.

Mendgen K, Struck C, Voegele RT, Hahn M. 2000. Biotrophy and rust haustoria. *Physiological and Molecular Plant Pathology* **56**: 141–145.

Mims CW, Celio GJ, Richardson EA. 2003. The use of high pressure freezing and freeze substitution to study host–pathogen interactions in fungal diseases of plants. *Microscopy and Microanalysis* **9**: 522–531.

Mims CW, Richardson EA, Holt IBF, Dangl JL. 2004. Ultrastructure of the host–pathogen interface in *Arabidopsis thaliana* leaves infected by the downy mildew *Hyaloperonospora parasitica*. *Canadian Journal of Botany* **82**: 1545–1545.

Murdoch LJ, Hardham AR. 1998. Components in the haustorial wall of the flax rust fungus, *Melampsora lini*, are labelled by three anti-calmodulin monoclonal antibodies. *Protoplasma* **201**: 180–193.

Narusaka Y, Narusaka M, Park P, Kubo Y, Hirayama T, Seki M, Shiraishi T, Ishida J, Nakashima M, Enju A, Sakurai T, Satou M, Kobayashi M, Shinozaki K. 2004. *RCH1*, a locus in *Arabidopsis* that confers resistance to the hemibiotrophic fungal pathogen *Colletotrichum higginsianum*. *Molecular Plant–Microbe Interactions* **17**: 749–762.

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC. 2003. Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science* **301**: 969–972.

Nomura K, Melotto M, He SY. 2005. Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Current Opinion in Plant Biology* **8**: 361–368.

Nühse TS, Boller T, Peck SC. 2003. A plasma membrane syntaxin is phosphorylated in response to the bacterial elicitor flagellin. *Journal of Biological Chemistry* **278**: 45 248–45 254.

Nürnberger T, Brunner F, Kemmerling B, Piater L. 2004. Innate immunity in plants and animals: striking similarities and obvious differences. *Immunological Reviews* **198**: 249–266.

Nürnberger T, Lipka V. 2005. Non-host resistance in plants: new insights into an old phenomenon. *Molecular Plant Pathology* **6**: 335–345.

O'Connell RJ. 1987. Absence of a specialized interface between intracellular hyphae of *Colletotrichum lindemuthianum* and cells of *Phaseolus vulgaris*. *New Phytologist* **107**: 725–734.

O'Connell RJ, Bailey JA, Richmond DV. 1985. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. *Physiological Plant Pathology* **27**: 75–98.

O'Connell R, Herbert C, Sreenivasaprasad S, Khatib M, Esquerre-Tugaye MT, Dumas B. 2004. A novel *Arabidopsis-Colletotrichum* pathosystem for the molecular dissection of plant–fungal interactions. *Molecular Plant–Microbe Interactions* **17**: 272–282.

O'Connell RJ, Ride JP. 1990. Chemical detection and ultrastructural localization of chitin in cell walls of *Colletotrichum lindemuthianum*. *Physiological and Molecular Plant Pathology* **37**: 39–53.

Panstruga R. 2003. Establishing compatibility between plants and obligate biotrophic pathogens. *Current Opinion in Plant Biology* **6**: 320–326.

Panstruga R. 2005. Serpentine plant MLO proteins as entry portals for powdery mildew fungi. *Biochemecial Society Transactions* **33**: 389–392.

Parniske M. 2000. Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? *Current Opinion in Plant Biology* **3**: 320–328.

Perfect SE, O'Connell RJ, Green EF, Doering-Saad C, Green JR. 1998. Expression cloning of a fungal proline-rich glycoprotein specific to the biotrophic interface formed in the *Colletotrichum*–bean interaction. *Plant Journal* **15**: 273–279.

Perfect SE, Pixton KL, O'Connell RJ, Green JR. 2000. The distribution and expression of a biotrophy-related gene, *CIH1*, within the genus *Colletotrichum*. *Molecular Plant Pathology* **1**: 213–221.

Pryce-Jones E, Carver T, Gurr SJ. 1999. The roles of cellulase enzymes and mechanical force in host penetration by *Erysiphe graminis* f.sp. *hordei*. *Physiological and Molecular Plant Pathology* **55**: 175–182.

Ramonell K, Berrocal-Lobo M, Koh S, Wan JR, Edwards H, Stacey G, Somerville S. 2005. Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. *Plant Physiology* **138**: 1027–1036.

Randall TA, Dwyer RA, Huitema E, Beyer K, Cvitanich C, Kelkar H, Fong A, Gates K, Roberts S, Yatzkan E, Gaffney T, Law M, Testa A, Torto-Alalibo T, Zhang M, Zheng L, Mueller E, Windass J, Binder A, Birch PRJ, Gisi U, Govers F, Gow NA, Mauch F, van West P, Waugh ME, Yu J, Boller T, Kamoun S, Lam ST, Judelson HS. 2005. Large-scale gene discovery in the oomycete *Phytophthora infestans* reveals likely components of phytopathogenicity shared with true fungi. *Molecular Plant–Microbe Interactions* **18**: 229–243.

Read ND, Kellock LJ, Collins TJ, Gundlach AM. 1997. Role of topography sensing for infection structure differentiation in cereal rust fungi. *Planta* **202**: 163–170.

Rehmany AP, Gordon A, Rose LE, Allen RL, Armstrong MR, Whisson SC, Kamoun S, Tyler BM, Birch PRJ, Beynon JL. 2005. Differential

recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two *Arabidopsis* lines. *Plant Cell* **17**: 1839–1850.

Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences, USA* **91**: 11841–11843.

Rep M. 2005. Small proteins of plant-pathogenic fungi secreted during host colonization. *FEMS Microbiology Letters* **253**: 19–27.

Ridge RW, Uozumi Y, Plazinski J, Hurley UA, Williamson RE. 1999. Developmental transitions and dynamics of the cortical ER of Arabidopsis cells seen with green fluorescent protein. *Plant and Cell Physiology* **40**: 1253–1261.

Ridout C. 2004. How powdery mildew becomes virulent. *11th International Cereal Rusts & Powdery Mildews Conference, Norwich, UK.* www.crpmb.org/icrpmc11/abstracts.htm (Abstract A1.45.)

Robaglia C, Caranta C. 2006. Translation initiation factors: a weak link in plant RNA virus infection. *Trends in Plant Science* **11**: 40–45.

Roberts AM, Mackie AJ, Hathaway V, Callow JA, Green JR. 1993. Molecular differentiation in the extrahaustorial membrane of pea powdery mildew haustoria at early and late stages of development. *Physiological and Molecular Plant Pathology* **43**: 147–160.

Schirawski J, Bohnert HU, Steinberg G, Snetselaar K, Adamikowa L, Kahmann R. 2005. Endoplasmic reticulum glucosidase II is required for pathogenicity of *Ustilago maydis*. *Plant Cell* **17**: 3532–3543.

Schmelzer E. 2002. Cell polarization, a crucial process in fungal defence. *Trends in Plant Science* **7**: 411–415.

Schulze-Lefert P. 2004. Knocking on heaven's wall: pathogenesis of and resistance to biotrophic fungi at the cell wall. *Current Opinion in Plant Biology* **7**: 377–383.

Shan WX, Cao M, Dan LU, Tyler BM. 2004. The *Avr1b* locus of *Phytophthora sojae* encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene *Rps1b*. *Molecular Plant–Microbe Interactions* **17**: 394–403.

Shen SH, Goodwin P, Hsiang T. 2001. Hemibiotrophic infection and identity of the fungus, *Colletotrichum destructivum*, causing anthracnose of tobacco. *Mycological Research* **105**: 1340–1347.

Shimada C, Lipka V, O'Connell R, Okuno T, Schulze-Lefert P, Takano Y. 2006. Nonhost resistance in *Arabidopsis–Colletotrichum* interactions acts at the cell periphery and requires actin filament function. *Molecular Plant–Microbe Interactions* **19**: 270–279.

Siegrist J, Kauss H. 1990. Chitin deacetylase in cucumber leaves infected by *Colletotrichum lagenarium*. *Physiological and Molecular Plant Pathology* **36**: 267–275.

Smith SE, Smith FA. 1990. Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytologist* **114**: 1–38.

Soanes DM, Talbot NJ. 2006. Comparative genomic analysis of phytopathogenic fungi using expressed sequence tag (EST) collections. *Molecular Plant Pathology* **7**: 61–70.

Soylu S. 2004. Ultrastructural characterisation of the host–pathogen interface in white blister-infected *Arabidopsis* leaves. *Mycopathologia* **158**: 457–464.

Stark-Urnau M, Mendgen K. 1995. Sequential deposition of plant glycoproteins and polysaccharides at the host–parasite interface of *Uromyces vignae* and *Vigna sinensis* – evidence for endocytosis and secretion. *Protoplasma* **186**: 1–11.

Stein M, Dittgen J, Sanchez-Rodriguez C, Hou BH, Molina A, Schulze-Lefert P, Lipka V, Somerville S. 2006. *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *Plant Cell* **18**: 731–746.

Struck C, Ernst M, Hahn M. 2002. Characterization of a developmentally regulated amino acid transporter (AAT1p) of the rust fungus *Uromyces fabae*. *Molecular Plant Pathology* **3**: 23–30.

Struck C, Hahn M, Mendgen K. 1996. Plasma membrane H^+-ATPase activity in spores, germ tubes, and haustoria of the rust fungus *Uromyces viciae fabae*. *Fungal Genetics and Biology* **20**: 30–35.

Struck C, Siebels C, Rommel O, Wernitz M, Hahn M. 1998. The plasma membrane H^+-ATPase from the biotrophic rust fungus *Uromyces fabae*: Molecular characterization of the gene (*PMA1*) and functional expression of the enzyme in yeast. *Molecular Plant–Microbe Interactions* **11**: 458–465.

Takemoto D, Jones DA, Hardham AR. 2003. GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of *Arabidopsis* by oomycete pathogens. *Plant Journal* **33**: 775–792.

Timmerman GM, Frew TJ, Weeden NF, Miller AL, Goulden DS. 1994. Linkage analysis of *er-1*, a recessive *Pisum sativum* gene for resistance to powdery mildew fungus (*Erysiphe pisi* D.C.). *Theoretical and Applied Genetics* **88**: 1050–1055.

Torto TA, Li SA, Styer A, Huitema E, Testa A, Gow NAR, van West P, Kamoun S. 2003. EST mining and functional expression assays identify extracellular effector proteins from the plant pathogen *Phytophthora*. *Genome Research* **13**: 1675–1685.

Tsurushima T, Don LD, Kawashima K, Murakami J, Nakayashiki H, Tosa Y, Mayama S. 2005. Pyrichalasin H production and pathogenicity of *Digitaria*-specific isolates of *Pyricularia grisea*. *Molecular Plant Pathology* **6**: 605–613.

Tucker SL, Talbot NJ. 2001. Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual Review of Phytopathology* **39**: 385–417.

Tudzynski P, Scheffer J. 2004. *Claviceps purpurea*: molecular aspects of a unique pathogenic lifestyle. *Molecular Plant Pathology* **5**: 377–388.

Urban M, Bhargava T, Hamer JE. 1999. An ATP-driven efflux pump is a novel pathogenicity factor in rice blast disease. *EMBO Journal* **18**: 512–521.

Van Damme M, Andel A, Huibers RP, Panstruga R, Weisbeek PJ, Van den Ackerveken G. 2005. Identification of *Arabidopsis* loci required for susceptibility to the downy mildew pathogen *Hyaloperonospora parasitica*. *Molecular Plant–Microbe Interactions* **18**: 583–592.

Voegele RT, Hahn M, Lohaus G, Link T, Heiser I, Mendgen K. 2005. Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiology* **137**: 190–198.

Voegele RT, Mendgen K. 2003. Rust haustoria: nutrient uptake and beyond. *New Phytologist* **159**: 93–100.

Voegele RT, Struck C, Hahn M, Mendgen K. 2001. The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proceedings of the National Academy of Sciences, USA* **98**: 8133–8138.

Voegele RT, Wirsel S, Möll U, Lechner M, Mendgen K. 2006. Cloning and Characterization of a novel invertase from the obligate biotroph *Uromyces fabae* and analysis of expression patterns of host and pathogen invertases in the course of infection. *Molecular Plant–Microbe Interactions*. (In press.)

Vogel JP, Raab TK, Schiff C, Somerville SC. 2002. *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in Arabidopsis. *Plant Cell* **14**: 2095–2106.

Vogel JP, Raab TK, Somerville CR, Somerville SC. 2004. Mutations in *PMR5* result in powdery mildew resistance and altered cell wall composition. *Plant Journal* **40**: 968–978.

Vogel J, Somerville S. 2000. Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences, USA* **97**: 1897–1902.

Vorwerk S, Somerville S, Somerville C. 2004. The role of plant cell wall polysaccharide composition in disease resistance. *Trends in Plant Science* **9**: 203–209.

Wang D, Weaver ND, Kesarwani M, Dong XN. 2005. Induction of protein secretory pathway is required for systemic acquired resistance. *Science* **308**: 1036–1040.

Wharton PS, Julian AM, O'Connell RJ. 2001. Ultrastructure of the infection of *Sorghum bicolor* by *Colletotrichum sublineolum*. *Phytopathology* **91**: 149–158.

Winnenburg R, Baldwin TK, Urban M, Rawlings C, Köhler J, Hammond-Kosack KE. 2006. PHI-base, a new database for pathogen host interactions. *Nucleic Acids Research* **34**: D459–D464.

Woods AM, Didehvar F, Gay JL, Mansfield JW. 1988. Modification of the host plasmalemma in haustorial infections of *Lactua sativa* by *Bremia lactucae*. *Physiological and Molecular Plant Pathology* **33**: 299–310.

Woods AM, Gay JL. 1983. Evidence for a neckband delimiting structural and physiological regions of the host plasma membrane associated with haustoria of *Albugo candida*. *Physiological Plant Pathology* **23**: 73–88.

Xu HX, Mendgen K. 1994. Endocytosis of 1,3-beta-glucans by broad bean cells at the penetration site of the cowpea rust fungus (haploid stage). *Planta* **195**: 282–290.

Xu HX, Mendgen K. 1997. Targeted cell wall degradation at the penetration site of cowpea rust basidiosporelings. *Molecular Plant–Microbe Interactions* **10**: 87–94.

Xu QL, Reed JC. 1998. Bax inhibitor-1, a mammalian apoptosis suppressor identified by functional screening in yeast. *Molecular Cell* **1**: 337–346.

Zeyen RJ, Carver TLW, Lyngkjaer MF. 2002. Epidermal cell papillae. In: Belanger RR, Bushnell WR, Dik AJ, Carver TLW, eds. *The powdery mildews, a comprehensive treatise*. St Paul, MN, USA: APS Press, 107–125.

Zhang Z, Henderson C, Gurr SJ. 2004. *Blumeria graminis* secretes an extracellular catalase during infection of barley: potential role in suppression of host defence. *Molecular Plant Pathology* **5**: 537–547.

Zhang Z, Henderson C, Perfect E, Carver TLW, Thomas BJ, Skamnioti P, Gurr SJ. 2005. Of genes and genomes, needles and haystacks: *Blumeria graminis and* functionality. *Molecular Plant Pathology* **6**: 561–575.

